US EPA ARCHIVE DOCUMENT

 Attachment #4

ANALYTICAL METHOD GUIDELINE 171-4

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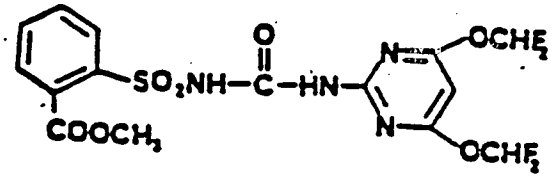
EDITION 1/16/87		DETERMINATION OF CGA-136872 IN DAIRY AND POULTRY TISSUES, EGGS AND MILK BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

1.0 SCOPE

This method is used for the determination of residues of CGA-136872 in poultry tissues (lean meat, skin plus adhering fat, liver and fat), eggs, dairy cow blood and tissues (round, loin, kidney, liver, perirenal fat and omental fat) and whole milk. The limit of determination for the method as established by the lowest fortification level is 0.01 ppm of CGA-136872 in milk and 0.05 ppm in the other substrates. The structure of CGA-136872 is shown below.



CGA-136872

2.0 PRINCIPLE

Parent residues of CGA-136872 are extracted from dairy and poultry tissues, eggs and milk by homogenizing weighed samples in 90% methanol/water for one minute using a Polytron homogenizer. The extract is filtered after addition of diatomaceous earth, then an aliquot is removed and partitioned with hexane. The methanol/water layer is evaporated to a small volume, diluted with a solution of sodium carbonate (0.1 M) and sodium chloride (2.0 M) then partitioned with ethyl acetate. After adding hexane, the ethyl acetate is partitioned several times with water/saturated sodium chloride/concentrated ammonium hydroxide, 50:2:1. The aqueous layers are combined, acidified with 10% acetic acid and partitioned with dichloromethane. The dichloromethane is evaporated, acetonitrile is added and the evaporation process repeated to remove any residual water. Final cleanup is performed with an Alumina-A Sep-Pak. Residues of CGA-136872 in all substrates, except milk, are determined by HPLC on a

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Zorbax-ODS column using a mobile phase comprised of 56% acetonitrile and 44% phosphate buffer with UV detection at 234 nm. For the determination of CGA-136872 residues in milk a mobile phase comprised of 54% acetonitrile and 46% phosphate buffer was used. A flow diagram for the method is presented in Figure 1.

3.0 APPARATUS

3.1 Bottle, Boston round, 8-oz.
3.2 Bottle, Nalgene (polyethylene), 8-oz. wide-mouth.
3.3 Bottle, square amber glass, 16 oz.
3.4 Centrifuge (Sorvall RC2-B, equipped with a Type GSA rotor or equivalent).
3.5 Filter paper, Whatman 2V, 24-cm.
3.6 Filter paper, Reeve Angel Grade 802, 24-cm.
3.7 Flasks, round bottom, 50-ml, 100-ml, 500-ml.
3.8 Funnel, filter, 10-cm.
3.9 Funnels, separatory, 125-ml and 250-ml.
3.10 Graduated cylinder, 100-ml.
3.11 Polytron Homogenizer, Brinkmann Instruments or equivalent.
3.12 Rotary Evaporator, Büchi Instruments or equivalent.
3.13 Sep-Pak, Alumina-A, Waters Assoc.
3.14 Syringes, Luer-Lok, 20-ml.
3.15 Ultrasonic Cleaner, Branson or equivalent.
3.16 Glass microvials, Wheaton Micro Product V Vials or equivalent.
3.17 Blender, Waring or equivalent, equipped with stainless steel container.

4.0 REAGENTS

4.1 Acetic acid, glacial, reagent grade.
4.2 Acetic acid:distilled water, 1:9
4.3 Acetonitrile, HPLC grade.
4.4 Ammonium hydroxide, concentrated (28-30%), reagent grade.
4.5 Celite (diatomaceous earth), analytical grade. Fisher Scientific.
4.6 Dichloromethane, HPLC grade.

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4.7 Ethyl acetate, HPLC grade.
4.8 Hexanes, HPLC grade.
4.9 Methanol, HPLC grade.
4.10 Methanol:distilled water, 9:1.
4.11 Phosphoric acid, reagent grade, 0.02 M in deionized water.
4.12 Potassium dihydrogen phosphate, reagent grade, 0.02 M in deionized water.
4.13 Sodium chloride, reagent grade, saturated solution in distilled water.
4.14 Sodium carbonate, reagent grade, 0.1 M and sodium chloride, reagent grade, 2.0 M in distilled water.
4.15 Methanol:acetonitrile, 15:85.
4.16 Toluene, 99 Mol % pure.
4.17 Distilled water:saturated sodium chloride:concentrated ammonium hydroxide, 50:2:1.
4.18 Distilled water:acetonitrile, 1:1
4.19 Standard CGA-136872 (available from CIBA-GEIGY Corp., P.O. Box 18300, Greensboro, NC 27419).

5.0 PROCEDURE

5.1 Sample Preparation

Beef and poultry meat, organ and fat samples are prepared by taking thin slices of the tissue from various sections of a partially frozen sample. The slices are then chopped into small pieces and mixed thoroughly before subsampling. Blood, eggs and milk are sampled directly after thawing. The milk and eggs are homogenized with the Polytron for a few seconds before the sample is withdrawn. Chicken skin, plus adhering fat, is prepared by placing alternate layers of crushed dry ice and skin in a shallow pan. The pan is covered with aluminum foil and allowed to stand for 15-30 minutes at which time the frozen chicken skin is removed, cut into smaller pieces and combined in a blender with slightly less than an equal portion of dry ice. After blending for several minutes, the mixture is poured into a plastic bag, loosely sealed and placed in a freezer until all of the carbon dioxide sublimes.

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5.2 Extraction

5.2.1 Weigh a 20-gram subsample of poultry tissue, dairy tissue, eggs, blood or milk into a 16-oz square amber glass bottle.

5.2.2 Add 200 ml of methanol:water (9:1) extraction solvent and homogenize for one minute with a Polytron homogenizer using the maximum power at which the sample will not splash out of the container.

5.2.3 Add 1 gram of diatomaceous earth, swirl and filter through Reeve Angel Grade 802 paper placed inside a Whatman 2V filter paper. Collect the filtrate in an 8-oz. Boston round bottle.

5.3 Partitions

5.3.1 Transfer a 50-ml aliquot (5-gram equivalent) of the extract from Step 5.2.3 into a 125-ml separatory funnel and partition with hexane (2 x 50 ml). Use a 100-ml aliquot of the extract and a 250-ml separatory funnel for the milk analysis. After the first partition, collect the lower layer in a 500-ml round bottom flask. Collect the lower layer after the second partition in the same round bottom flask, and add 30 ml of toluene to the flask.

5.3.2 On a rotary evaporator (bath temperature set at 40°C) evaporate the solvent from the 500-ml round bottom flask until the toluene and methanol stop distilling, at which point there will be 5 - 10 ml of liquid remaining in the flask. Add 40 ml of 0.1 M sodium carbonate/2.0 M sodium chloride solution to the 500-ml round bottom flask, swirl several times and transfer the contents to a 125-ml separatory funnel.

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5.3.3 Add 30 ml of ethyl acetate to the 125-ml separatory funnel from Step 5.3.2. Shake the separatory funnel gently for one minute, then, after the layers separate, drain the lower layer back into the 500-ml round bottom flask from Step 5.3.2 and pour the upper organic layer from the top of the 125-ml separatory funnel into a 250-ml separatory funnel. (With some substrates an emulsion will form during this partition. If this occurs, drain most of the non-emulsified lower layer then stir the emulsion rapidly with a glass rod and allow the layers to separate. Again, drain most of the non-emulsified lower layer and, if necessary, restir. Repeat this process until only a small emulified layer remains. In any case, drain the persisting emulsion out of the separatory funnel before transferring the upper layer. Alternatively, the emulsion can be broken by transferring the entire contents of the separatory funnel to an 8-oz. Nalgene bottle and centrifuging at 5000 rpm for ten minutes. After centrifuging the two layers are poured slowly back into the original separatory funnel and the separation is continued.)

5.3.4 Swirl the contents of the 500-ml round bottom flask and pour back into the 125-ml separatory funnel used for the first partition. Add 30 ml of ethyl acetate and partition again as in Step 5.3.3.

5.3.5 To the 250-ml separatory funnel, which contains the combined organic layers from Steps 5.3.3 and 5.3.4, add 30 ml of hexane.

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5.3.6 Partition the contents of the 250-ml separatory funnel by shaking gently for one minute with three 40-ml portions of a distilled water:sat. sodium chloride:conc. ammonium hydroxide, 50:2:1 mixture. (All substrates will separate into two distinct layers after this partition, but in some cases, a finely dispersed emulsion will persist in the lower, aqueous layer. If this occurs, vigorous stirring will often cause the emulsion to dissipate. In some substrates the samples will have to be centrifuged as described in Section 5.3.3.)

5.3.7 Combine the lower layers from Step 5.3.6 in a second 250-ml separatory funnel, acidify by adding 20 ml of 10% acetic acid, then partition with two 25-ml portions of dichloromethane by shaking vigorously for 30 seconds.

5.3.8 Collect and combine the lower, dichloromethane layers in a 100-ml round bottom flask and evaporate the solvent on a rotary evaporator (bath temperature at 40°C). As soon as no solvent is visible, stop the evaporation, add 5 ml of acetonitrile, swirl the flask, and evaporate again in order to azeotropically remove any traces of water. It is important not to leave the flask on the rotary evaporator for prolonged time periods after the solvent evaporates.

5.4 Cleanup

5.4.1 Connect the inlet of an Alumina-A Sep-Pak to a 20-ml Luer-Lok syringe.

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5.4.2 Prewash the Sep-Pak with 5 ml of 85% acetonitrile/methanol. It may be necessary to start the solvent flow through the Sep-Pak by applying pressure with a pipette bulb or pressurized air. Once the flow is started, allow the solvent to drain by gravity. Discard the wash solvent.

5.4.3 Dissolve the residue in the 100-ml round bottom flask from Step 5.3.8 in 5 ml of 85% acetonitrile/methanol, sonicate and pipette into the syringe. Start the flow as before. Collect the eluant in a 50-ml round bottom flask.

5.4.4 Add 5 ml of 85% acetonitrile/methanol to the 100-ml round bottom flask from Step 5.4.3, swirl thoroughly and pipette the solution into the syringe after the first 5 ml of solvent has stopped flowing.

5.4.5 Add an additional 10 ml of 85% acetonitrile/methanol to the flask from Step 5.4.4 and swirl. After rinsing the pipette used for sample transfer, pour the solution into the syringe. When elution of the Sep-Pak is complete, remove the 50-ml round bottom flask and evaporate the solvent to dryness on a rotary evaporator (bath temperature 40°C).

5.4.6 To the 50-ml flask add 1.0 ml of acetonitrile/water (1:1), or multiples of 1.0 ml for higher residue levels, and dissolve the residue by sonicating. Use 0.5 ml of acetonitrile/water (1:1) for milk.

5.4.7 Pipette the sample into a standard 1-ml glass vial for HPLC analysis. Use a glass microvial for milk samples.

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6.0 HPLC ANALYSIS

6.1 Preparation of Standard CGA-136872

6.1.1 Weigh 100.0 mg of CGA-136872 analytical standard into a 100-ml volumetric flask and dilute the flask to the mark with acetonitrile.

6.1.2 Make serial dilutions of the 1 mg/ml standard solution with acetonitrile/water (1:1) to give a series of injection standards in a range of 0.1 to 5.0 ng per µl.

6.2 Standardization

6.2.1 Standardize the HPLC under the conditions listed in Table I by making 20-ul injections in the range of 2 to 100 ng.

6.2.2 Measure the peak heights of the injected standards. Typical chromatograms for standards are shown in Figure 2 and standardization data generated from the chromatograms are listed in Table II.

6.2.3 Construct a standard curve by plotting, either manually or by computer, the detector response versus nanograms injected, or enter the data into an appropriate electronic calculator to obtain a least squares regression.

6.3 Detection of Sample Residues

6.3.1 Inject a 20-ul aliquot of the sample from Step 5.4.7 into the HPLC under the same conditions employed for standards. Make dilutions of samples, as necessary, to maintain peak heights within the range of the standard curve. Compare the peak heights of the unknown samples with the standard curve or

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enter into the least squares program to determine the nanograms of CGA-136872 present in the injected aliquot.

6.3.2 Calculate the residue results in terms of ppm CGA-136872 by the following equation:

$$\text{ppm CGA-136872} = \frac{\text{ng CGA-136872 found}}{\text{mg sample injected}} \times \frac{100}{R}$$

where mg sample injected is calculated as follows:

$$\frac{\text{grams of sample}}{\text{total extract volume (ml) including volume increase due to sample moisture}} \times \text{aliquot volume (ml)} \times \frac{\text{injection volume } (\mu l)}{\text{total volume in final fraction } (\mu l)}$$

$$\times\ 1000 = \text{mg sample injected}$$

Alternatively, the residue results can be calculated by the following equation:

$$\text{ppm CGA-136872} = \frac{\text{ng CGA-136872 found}}{\text{apparent mg sample injected}} \times \frac{V+(WxM/100)}{V} \times \frac{100}{R}$$

where apparent mg sample injected does not account for volume increase due to sample moisture. R is the recovery factor (%) determined using fortified control samples carried through the procedure, V is the volume of extraction solvent, W is the weight of the sample (grams) and M is the percent moisture in the substrate. The percent moisture for the various substrates is taken from the PAM, Vol.1, Section 202[1]. The moisture contents of

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meat, blood and organs were taken as 80%. No moisture correction was made for fat samples and chicken skin.

6.4 Fortification Experiments

This method is validated for each set of samples analyzed by including an untreated control sample and one or more control samples fortified prior to extraction with 0.05 ppm or more of CGA-136872. Control milk samples are fortified with 0.01 ppm or more of CGA-136872.

6.4.1 Add 1.00 ml of 1 ug/ml standard solution of CGA-136872 in acetonitrile/water (1:1) to 20 g of control sample prior to homogenization (Step 5.2.2) for a 0.05 ppm fortification. Use correspondingly greater amounts of standards (volume not to exceed 1 ml) for higher fortifications. Analyze the control and fortified samples by the procedures of the method. Typical chromatograms from each substrate are shown in Figures 3 to 15. Recovery data are summarized in Table III.

6.4.2 Calculate the final ppm value for the control and fortified samples according to either of the following equations:

$$\text{ppm CGA-136872} = \frac{\text{ng CGA-136872 found}}{\text{mg sample injected}}$$

$$\text{ppm CGA-136872} = \frac{\text{ng CGA-136872 found}}{\text{apparent mg injected}} \times \frac{V+(W \times M/100)}{V}$$

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where mg sample injected and apparent mg sample injected are calculated as described in Section 6.3.2. The letters V, M and W have the same significance as in Section 6.3.2.

6.4.3 Determine the recovery factor by first subtracting the response, if any, in the control sample from the CGA-136872 response in the fortified sample. Then calculate the recovery factor in percent by the following equation:

$$R(\%) = \frac{\text{ppm found (corrected)}}{\text{ppm added}} \times 100$$

7.0 TIME REQUIRED FOR ANALYSIS

A set of six samples can be fortified, extracted, partitioned and prepared for HPLC injection in an eight hour period. The time required for the final HPLC determination will vary with individual substrates but typically run times of about twenty minutes are adequate. For convenience, or in the case of large sample sets which cannot be completely analyzed in one day, the samples can be weighed, fortified and extracted, and the extracts can be stored for several weeks at refrigerator temperatures before analysis. After four weeks of storage significant decomposition of CGA-136872 can be detected in some substrates so it is recommended that extracts be stored no longer than two weeks prior to analysis. The analysis can be interrupted and samples stored for several days in the freezer after the final partition (Step 5.3.8) into dichloromethane (either before or after removing the dichloromethane) and also after the Sep-Pak cleanup (Step 5.4.5). The partitions (Steps 5.3.1 to 5.3.8) should be carried out on the same day.

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8.0 POTENTIAL PROBLEMS

In milk the HPLC mobile phase had to be changed to 54% acetonitrile/46% phosphate buffer in order to completely resolve a small, unidentified peak (Figure 3, peak A) from the CGA-136872 peak.

In the acid form when dissolved in mixtures of acetonitrile/water, CGA-136872 adsorbs to plastic surfaces, so glass microvials must be used for the HPLC determination and filtration of samples prior to HPLC injection using filters with plastic housings is not advisable.

9.0 DISCUSSION

This method has been used for the analysis of control and fortified control samples of poultry tissues, dairy blood and tissues, eggs and milk. Fortification levels ranged from 0.05 ppm (0.01 ppm in milk) to 1.00 ppm and recoveries averaged 91% with a standard deviation of 6.5% (n=56). At the screening level of 0.05 ppm (0.01 ppm in milk) the average recovery was 91% with a standard deviation of 8.1% (n=27). No residues at or above the screening level were found in any of the control samples. Periodically, reagent blanks were run along with sample sets to check for interference from solvents and labware. Chromatograms from blank runs showed no interfering peaks. Results of these analyses are reported in AG-A 9871-01.

This method has also been used for the analysis of goat liver from a metabolism study[2] in which a lactating goat was dosed eleven days consecutively with ϕ-^{14}C-CGA-136872 at a level equivalent to approximately 4 ppm in the feed (results are summarized in Table IV). In the metabolism study the contribution from parent CGA-136872 to the total residue was reported as approximately 33%. Method AG-506 accounted for 21% (0.03 ppm) of the total residue as CGA-136872. Results of these analyses are reported in AG-A 9870-01.

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REFERENCES

1. Pesticide Analytical Manual, Volume I, "Methods Which Detect Multiple Residues," U.S. Department of Health and Human Services, Food and Drug Administration.

2. W. A. Anderson, S. O. Madrid and J. E. Cassidy, ABR-85076, "Metabolism of Phenyl-^{14}C-CGA-136872 by a Lactating Goat Dosed at 4 ppm for Eleven Consecutive Days".

3. M. Torbett, SOP No. 4.67, "Operation, Maintenance and Calibration of Manual Harvey OX-400 Oxidizers".

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TABLE I: HPLC OPERATING CONDITIONS FOR DETERMINATION OF CGA-136872

Instrument:	Perkin-Elmer Series 4 Liquid Chromatograph with a LC85B Variable Wavelength UV Detector, an ISS-100 Sampling System and a Chromatographics 3 Data Handling System or an equivalent HPLC pump and variable wavelength UV detector with or without automated data acquisition.
Column:	Zorbax-ODS, 4.6 x 250 mm (Dupont Instruments)
Mobile phase:*	56% acetonitrile/44% phosphate buffer solution (0.02 M potassium dihydrogen phosphate:0.02 M phosphoric acid, 4:1, pH = 2.8)
Flow Rate:	1.0 ml/min.
Column Temperature:	35°C
Attenuation:	0.02 AUFS
Detection:	Variable wavelength UV detector set at 234 nm
Limit of Detection:	2.0 ng
Injection volume:	20 ul
Chart Speed:	5 mm/min.
Retention Time:	9 min.

*In milk it was necessary to adjust the mobile phase to 54% acetonitrile/46% phosphate buffer in order to completely resolve the CGA-136872 peak from a small, unidentified peak (Figure 3, peak A).

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TABLE II: TYPICAL STANDARDIZATION DATA FOR CGA-136872 (AG-A 9871-01)

CGA-136872 Injected (ng)	Peak Height
2	0.9366
4	1.5724
10	4.1952
40	17.3587

Slope = 0.4354511 ht. units/ng

Intercept = -0.08059 ht. units

Correlation Coefficient = 0.9998988 (calculated by TI-55 calculator)

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TABLE III: SUMMARY OF RECOVERY DATA FOR MEAT, MILK AND EGG SAMPLES FORTIFIED WITH CGA-136872 (AG-A 9871-01)

Substrates	% Recoveries at Various Fortification Levels				
	0.01 ppm	0.05 ppm	0.10 ppm	0.20 ppm	1.00 ppm
Milk	91,96,75	87	92	95	
Blood		88,96	92	86	89
Dairy Loin		84,80	82	80	98
Dairy Round		106,97	101	85	87
Dairy Perirenal Fat		91,100	95	94	95
Dairy Omental Fat		110,89	95	95	97
Dairy Liver		96,92	94	91	93
Dairy Kidney		92,87	89	94	94
Chicken Lean Meat		89,94	91	92	89
Chicken Skin plus adhering fat		95,78	86	81	86
Chicken Fat		84,93	89	97	93
Chicken Liver		91,82	86	94	91
Eggs		90,79	84	87	89

Average recovery at the screening level of 0.05 ppm (0.01 ppm in milk) is 91% (S.D. = 8.1%, n=27).

Average recovery for all levels is 91% (S.D, = 6.5%, n=56).

No residues at or above the screening level of 0.05 ppm (0.01 ppm in milk) were found in any of the control samples.

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TABLE IV: ANALYSIS OF LIVER FROM GOAT DOSED WITH ϕ-^{14}C-CGA-136872[a]

AGA No.: 9870-01

Test No.: M6-161-5A

Location: CIBA-GEIGY Research Facility at Vero Beach, Florida

Sample: Goat #36, Liver(Rep B)

Total ppm[b]: 0.120

Results of analysis by AG-506

Percent of total ^{14}C in extract[c]: 87%

Percent (ppm) of total ^{14}C in final fraction[c]: 17% (0.021)

CGA-136872 in final fraction determined by HPLC:

Uncorrected for procedural recovery	0.02 ppm (18% of total residue)
Corrected for procedural recovery of 88%	0.03 ppm (21% of total residue)

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TABLE IV: ANALYSIS OF LIVER FROM GOAT DOSED WITH ϕ-^{14}C-CGA-136872[a] (continued)

a) One lactating goat was treated with eleven consecutive daily oral doses of ϕ-^{14}C-CGA-136872 at a level equivalent to approximately 4 ppm in the feed. Twenty-three hours after the last dose, the goat was sacrificed and samples of the tissues were collected. See reference 2 for details.

b) Total ppm determined by combustion and measurement of $^{14}CO_2$ in accordance with SOP No. 4.67[3].

c) Determined by liquid scintillation counting of aliquots of solutions.

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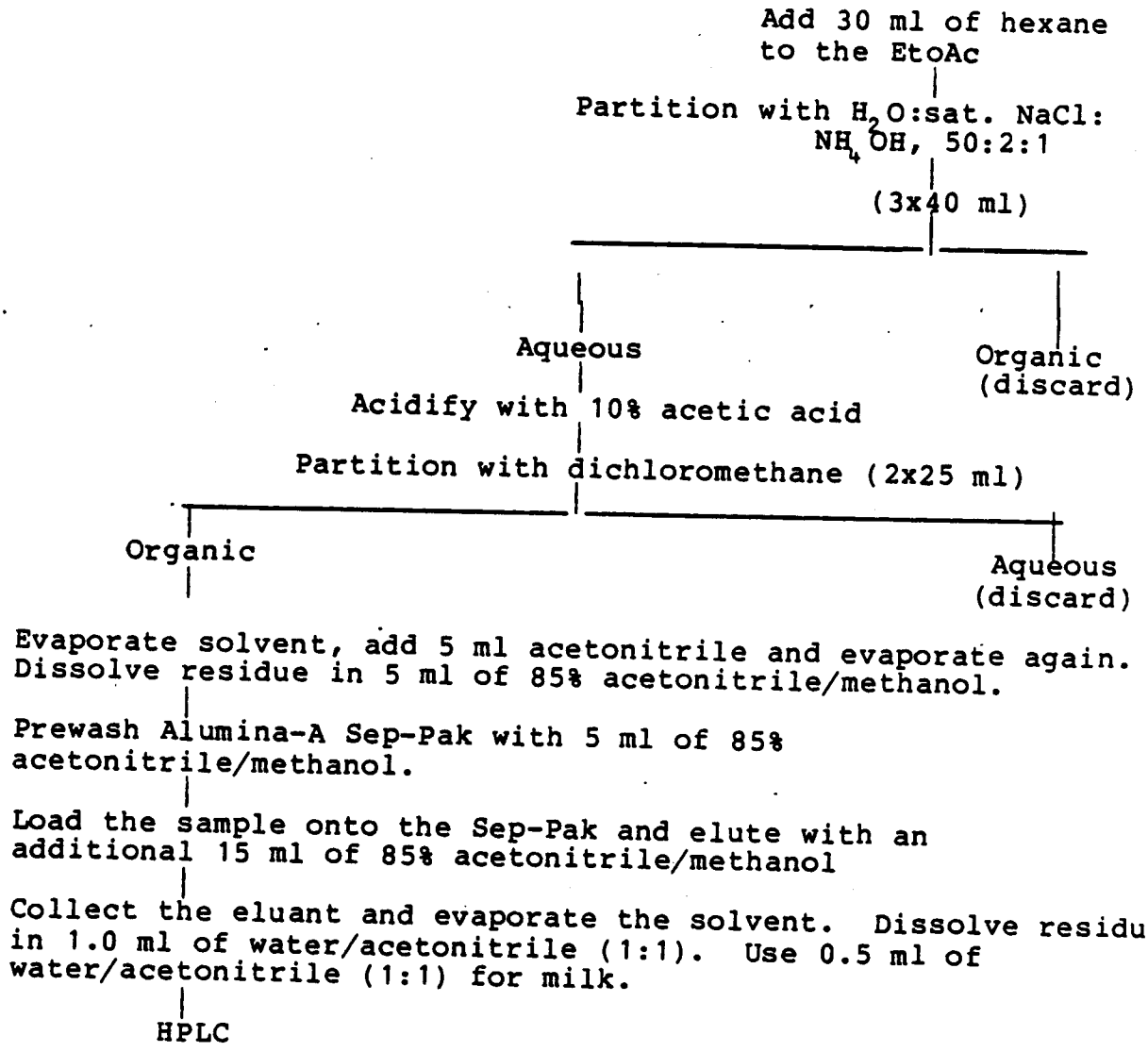
FIGURE 1: FLOW DIAGRAM OF ANALYTICAL METHOD AG-506
(continued)



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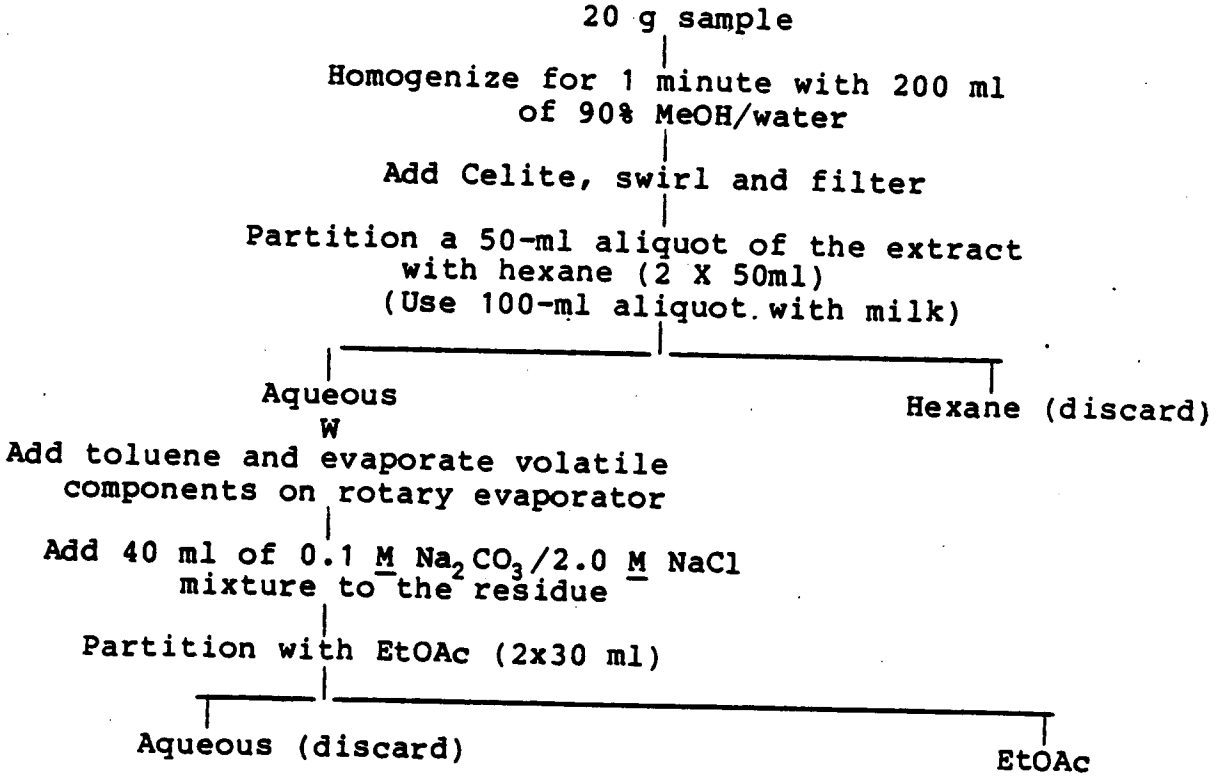
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

FIGURE 1: FLOW DIAGRAM OF ANALYTICAL METHOD AG-506



(over)

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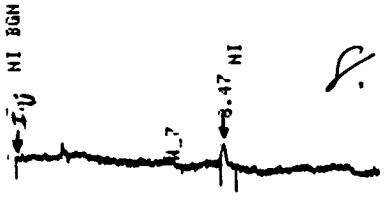
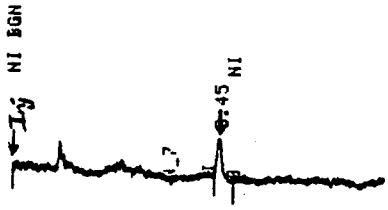
		APPROVED BY:

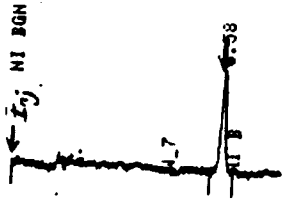
FIGURE 2: TYPICAL CHROMATOGRAMS OF CGA-136872 STANDARDS



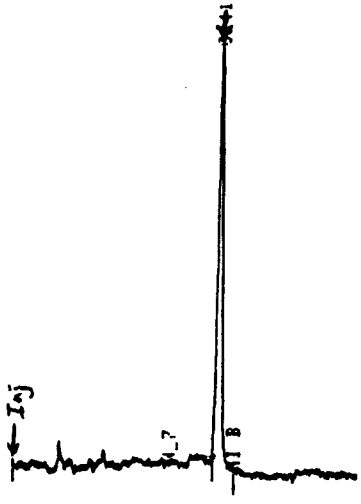
2 ng



4 ng



10 ng



40 ng

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SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

FIGURE 3: TYPICAL CHROMATOGRAMS FOR MILK

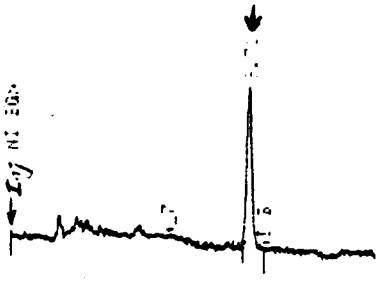
Control Milk*
400 mg injected
<2 ng CGA-136872
<0.01 ppm

Control + 0.01 ppm
400 mg injected
3.33 ng CGA-136872
91% recovery

Control + 0.01 ppm
400 mg injected
3.53 ng CGA-136872
96% recovery

Control + 0.01 ppm
400 mg injected
2.75 ng CGA-136872
75% recovery

Control + 0.10 ppm
200 mg injected
16.8 ng CGA-136872
92% recovery



Control 0.20 ppm
100 mg injected
17.5 ng CGA-136872
95% recovery

*Recoveries are corrected for 87% moisture content by formula in Section 6.4.2.

Note: See Section 8.0 for discussion of unidentified peak A.

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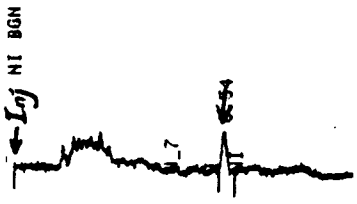
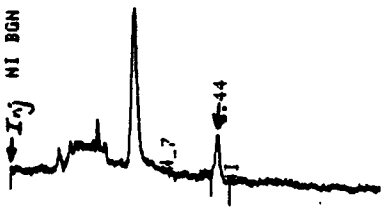
		APPROVED BY:

FIGURE 4: TYPICAL CHROMATOGRAMS FOR DAIRY BLOOD

Control Blood*
100 mg injected
<2 ng CGA-136872
<0.05 ppm



Control + 0.05 ppm
100 mg injected
4.10 ng CGA-136872
88% recovery



Control + 0.05 ppm
100 mg injected
4.42 ng CGA-136872
96% recovery

Control + 0.10 ppm
100 mg injected
8.00 ng CGA-136872
86% recovery

Control + 0.20 ppm
100 mg injected
16.5 ng CGA-136872
89% recovery

*Recoveries are corrected for 80% moisture content by formula in Section 6.4.2.

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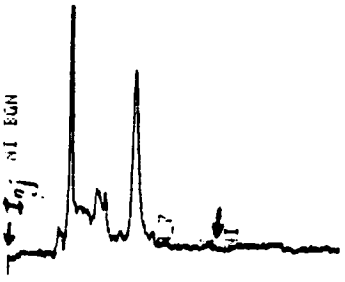
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

FIGURE 5: TYPICAL CHROMATOGRAMS FOR DAIRY LOIN MEAT

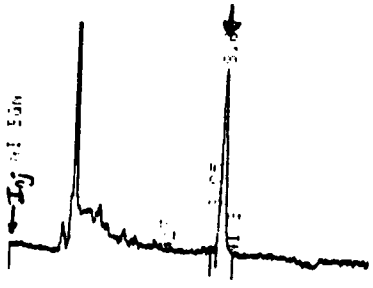


Control Loin*
100 mg injected
<2 ng CGA-136872
<0.05 ppm

Control + 0.05 ppm
100 mg injected
3.90 ng CGA-136872
84% recovery

Control + 0.05 ppm
100 mg injected
3.72 ng CGA-136872
80% recovery

Control + 0.10 ppm
100 mg injected
7.45 ng CGA-136872
80% recovery



Control + 0.20 ppm
100 mg injected
18.2 ng CGA-136872
98% recovery

*Recoveries are corrected for 80% moisture content by formula in Section 6.4.2.

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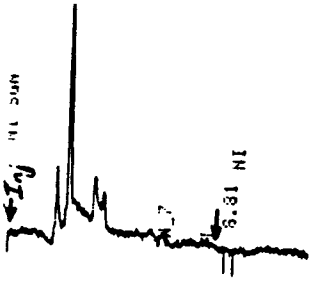
		APPROVED BY:

FIGURE 6: TYPICAL CHROMATOGRAMS FOR DAIRY ROUND MEAT

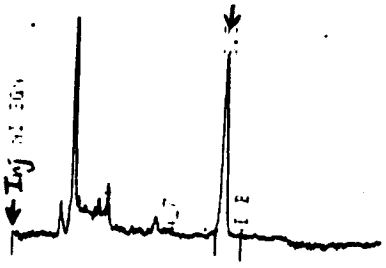


Control Round*
100 mg injected
<2 ng CGA-136872
<0.05 ppm

Control + 0.05 ppm
100 mg injected
4.91 ng CGA-136872
106% recovery

Control + 0.05 ppm
100 mg injected
4.51 ng CGA-136872
97% recovery

Control + 0.10 ppm
100 mg injected
7.90 ng CGA-136872
85% recovery



Control + 0.20 ppm
100 mg injected
16.2 ng CGA-136872
87% recovery

*Recoveries are corrected for 80% moisture content by formula in Section 6.4.2.

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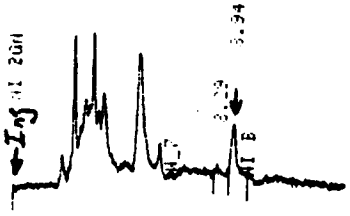
		APPROVED BY:

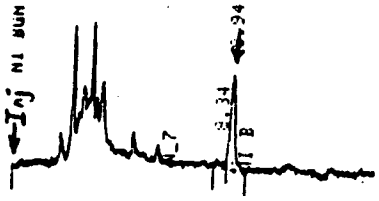
FIGURE 7: TYPICAL CHROMATOGRAMS FOR DAIRY LIVER

Control Liver*
100 mg injected
<2 ng CGA-136872
<0.05 ppm

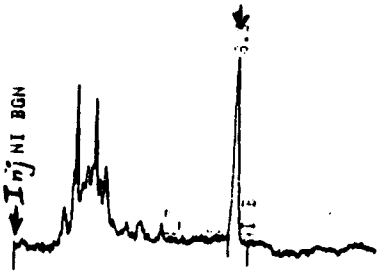


Control + 0.05 ppm
100 mg injected
4.46 ng CGA-136872
96% recovery

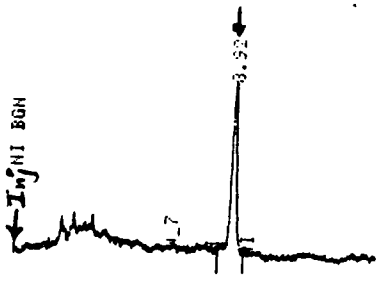
Control + 0.05 ppm
100 mg injected
4.25 ng CGA-136872
92% recovery



Control + 0.10 ppm
100 mg injected
8.43 ng CGA-136872
91% recovery



Control + 0.20 ppm
100 mg injected
17.3 ng CGA-136872
93% recovery



Control + 1.00 ppm
20 mg injected
16.2 ng CGA-136872
88% recovery

*Recoveries are corrected for 80% moisture content by formula in Section 6.4.2.

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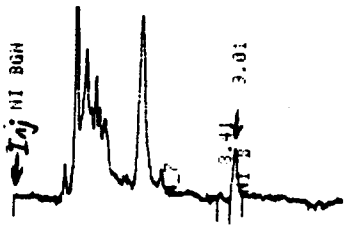
		APPROVED BY:

FIGURE 8: TYPICAL CHROMATOGRAMS FOR DAIRY KIDNEY

Control Kidney*
100 mg injected
<2 ng CGA-136872
<0.05 ppm

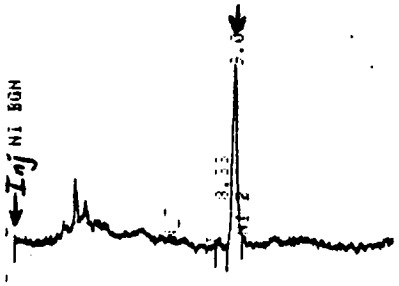
Control + 0.05 ppm
100 mg injected
4.28 ng CGA-136872
92% recovery



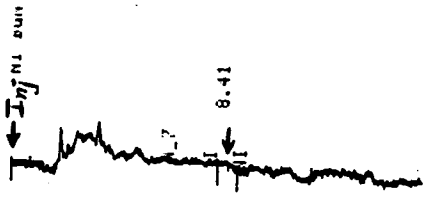
Control + 0.05 ppm
100 mg injected
4.03 ng CGA-136872
87% recovery

Control + 0.10 ppm
100 mg injected
8.71 ng CGA-136872
94% recovery

Control + 0.20 ppm
100 mg injected
17.4 ng CGA-136872
94% recovery



Control + 1.00 ppm
20 mg injected
16.5 ng CGA-136872
89% recovery



Reagent Blank
100 mg injected
<2 ng CGA-136872
<0.05 ppm

*Recoveries are corrected for 80% moisture content by formula in Section 6.4.2.

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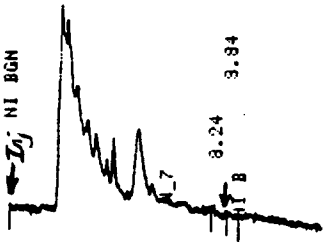
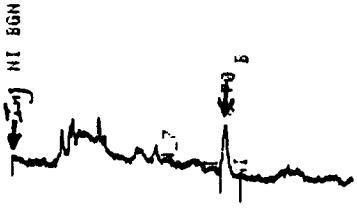
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

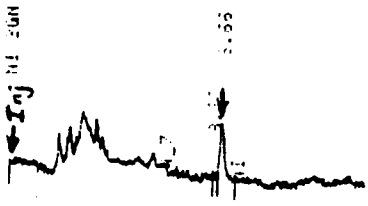
FIGURE 9: TYPICAL CHROMATOGRAMS FOR DAIRY PERIRENAL FAT



Control Fat
100 mg injected
<2 ng CGA-136872
<0.05 ppm



Control + 0.05 ppm
100 mg injected
4.54 ng CGA-136872
91% recovery



Control + 0.05 ppm
100 mg injected
4.99 ng CGA-136872
100% recovery

Control + 0.10 ppm
100 mg injected
9.37 ng CGA-136872
94% recovery

Control + 0.20 ppm
100 mg injected
18.9 ng CGA-136872
95% recovery

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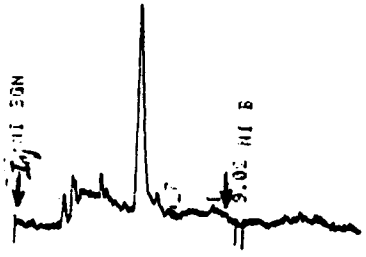
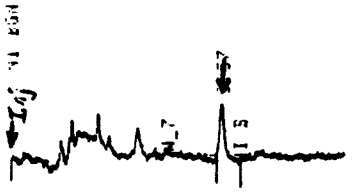
		APPROVED BY:

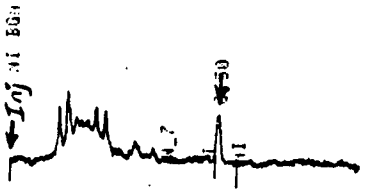
FIGURE 10: TYPICAL CHROMATOGRAMS FOR DAIRY OMENTAL FAT



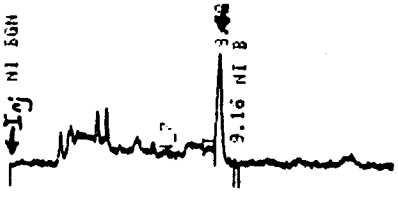
Control Fat
100 mg injected
<2 ng CGA-136872
<0.05 ppm



Control + 0.05 ppm
100 mg injected
5.49 ng CGA-136872
110% recovery



Control + 0.05 ppm
100 mg injected
4.47 ng CGA-136872
89% recvery



Control + 0.10 ppm
100 mg injected
9.51 ng CGA-136872
95% recovery

Control + 0.20 ppm
100 mg injected
19.4 ng CGA-136872
97% recovery

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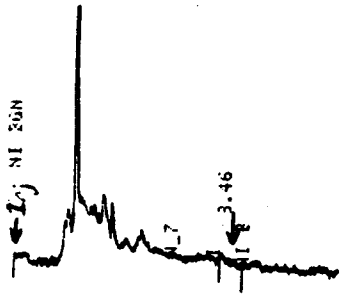
		APPROVED BY:

FIGURE 11: TYPICAL CHROMATOGRAMS FOR CHICKEN LEAN MEAT



Control Chicken Meat*
100 mg injected
<2 ng CGA-136872
<0.05 ppm

Control + 0.05 ppm
100 mg injected
4.11 ng CGA-136872
89% recovery

Control + 0.05 ppm
100 mg injected
4.35 ng CGA-136872
94% recovery

Control + 0.10 ppm
100 mg injected
8.54 ng CGA-136872
92% recovery

Control + 0.20 ppm
100 mg injected
16.4 ng CGA-136872
89% recovery

*Recoveries are corrected for 80% moisture content by formula in Section 6.4.2.

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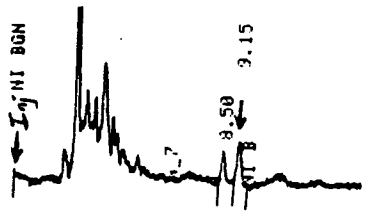
		APPROVED BY:

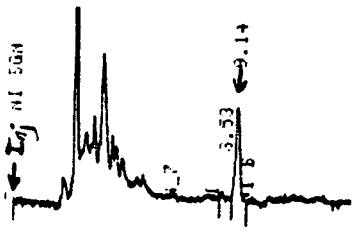
FIGURE 12: TYPICAL CHROMATOGRAMS FOR CHICKEN LIVER

Control Liver*
100 mg injected
<2 ng CGA-136872
<0.05 ppm

Control + 0.05 ppm
100 mg injected
4.23 ng CGA-136872
91% recovery



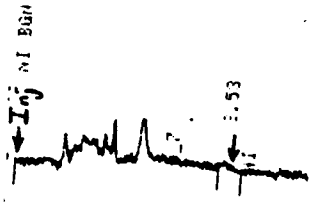
Control + 0.05 ppm
100 mg injected
3.80 ng CGA-136872
82% recovery



Control + 0.10 ppm
100 mg injected
8.67 ng CGA-136872
94% recovery

Control + 0.20 ppm
100 mg injected
16.9 ng CGA-136872
91% recovery

Control + 1.00 ppm
20 mg injected
17.2 ng CGA-136872
93% recovery



Reagent Blank
100 mg injected
<2 ng CGA-136872
<0.05 ppm

*Recoveries are corrected for 80% moisture content by formula in Section 6.4.2.

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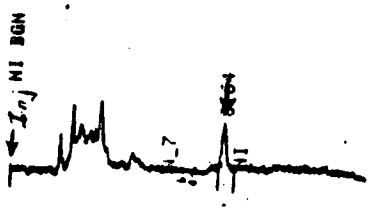
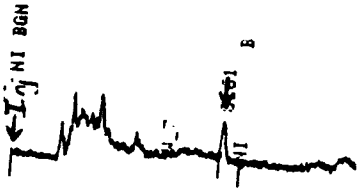
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

FIGURE 13: TYPICAL CHROMATOGRAMS FOR CHICKEN SKIN PLUS ADHERING FAT

Control skin
100 mg injected
<2 ng CGA-136872
<0.05 ppm



Control + 0.05 ppm
100 mg injected
4.73 ng CGA-136872
95% recovery



Control + 0.05 ppm
100 mg injected
3.90 ng CGA-136872
78% recovery

Control + 0.10 ppm
100 mg injected
8.11 ng CGA-136872
81% recovery

Control + 0.20 ppm
100 mg injected
17.1 ng CGA-136872
86% recovery

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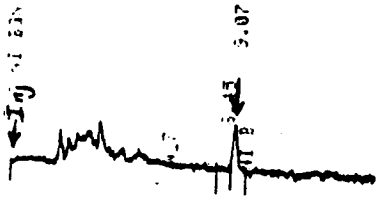
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

FIGURE 14: TYPICAL CHROMATOGRAMS FOR CHICKEN FAT

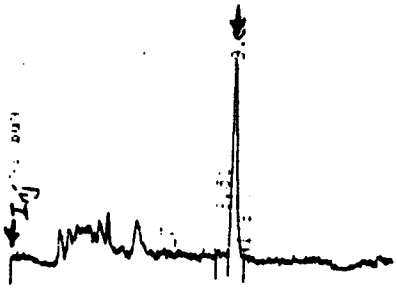
Control Fat
100 mg injected
<2 ng CGA-136872
<0.05 ppm



Control + 0.05 ppm
100 mg injected
4.21 ng CGA-136872
84% recovery

Control + 0.05 ppm
100 mg injected
4.64 ng CGA-136872
93% recovery

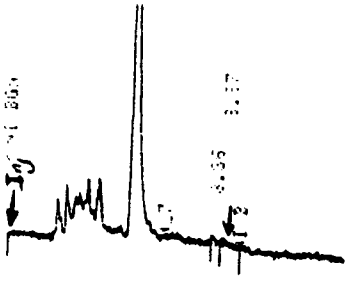
Control + 0.10 ppm
100 mg injected
9.67 ng CGA-136872
97% recovery



Control + 0.20 ppm
100 mg injected
18.6 ng CGA-136872
93% recovery

PAGE 34 of 34	METHOD NO. AG-506	SUBJECT
EDITION 1/16/87		DETERMINATION OF CGA-136872 IN DAIRY AND POULTRY TISSUES, EGGS AND MILK BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

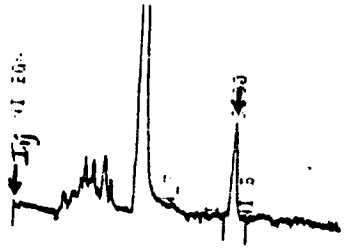
FIGURE 15: TYPICAL CHROMATOGRAMS FOR EGGS



Control Eggs*
100 mg injected
<2 ng CGA-136872
<0.05 ppm

Control + 0.05 ppm
100 mg injected
4.20 ng CGA-136872
90% recovery

Control + 0.05 ppm
100 mg injected
3.68 ng CGA-136872
79% recovery



Control + 0.10 ppm
100 mg injected
8.06 ng CGA-136872
87% recovery

Control + 0.20 ppm
100 mg injected
16.5 ng CGA-136872
89% recovery

*Recoveries are corrected for 74% moisture content by formula in Section 6.4.2.

PAGE 1 of 5	METHOD NO. AG-506A	SUBJECT
EDITION 7/30/87		ADDENDUM TO AG-506: SUBSTITUTION OF ACETONITRILE FOR METHANOL FOR EXTRACTION OF CGA-136872 RESIDUES FROM MILK
SUBMITTED BY: K. Van Geluwe-Barvir, T. Beidler		
		APPROVED BY:

1.0 SCOPE

This addendum describes the substitution of acetonitrile for methanol used in AG-506, "Determination of CGA-136872 in Dairy and Poultry Tissues, Eggs and Milk by High Performance Liquid Chromatography," for extraction with milk as the substrate. Both solvents are equally effective in extracting CGA-136872 but acetonitrile eliminates coextractives encountered with milk that cause emulsions when methanol is used. The use of diatomaceous earth is not necessary when extracting with acetonitrile. The extraction is performed by shaking for 20 minutes on a mechanical shaker.

This alternative solvent for extraction is a direct substitution. The ratio of acetonitrile:water remains the same as methanol:water at 9:1.

2.0 PRINCIPLE

The entire method showing the substitution of 90% acetonitrile/water for 90% methanol/water as the extracting solvent is outlined in Figure 1. Additional procedural details have been added to the pertinent Sections of AG-506 to provide better descriptions of the results using acetonitrile.

Only those sections dealing specifically with the extraction are listed in this addendum. The corresponding numbering system of AG-506 is used.

3.0 APPARATUS

3.18 Mechanical Shaker

4.0 REAGENTS

4.20 Acetonitrile:distilled water, 9:1.

PAGE 2 of 5	METHOD NO. AG-506A	SUBJECT
EDITION		ADDENDUM TO AG-506: SUBSTITUTION OF ACETONITRILE FOR METHANOL FOR EXTRACTION OF CGA-136872 RESIDUES FROM MILK
SUBMITTED BY: K. Van Geluwe-Barvir, T. Beidler		
		APPROVED BY:

5.0 PROCEDURE

5.2 Extraction

5.2.1 Milk

5.2.1.1 Weigh a 20-gram subsample of milk into a 16-oz. square amber glass bottle.

5.2.1.2 Add 200 ml of acetonitrile:water (9:1) extraction solvent. Cap bottle and shake for 20 minutes on a mechanical shaker.

9.0 DISCUSSION (of AG-506A)

9.1 Acetonitrile and H_2O (9:1) has been used as an alternative solvent for extraction of milk samples analyzed by AG-506. Representative HPLC chromatograms are illustrated in Figure 2.

Recovery data for CGA-136872 using this extraction technique are given below:

% Recovery at Various Fortifications of CGA-136872

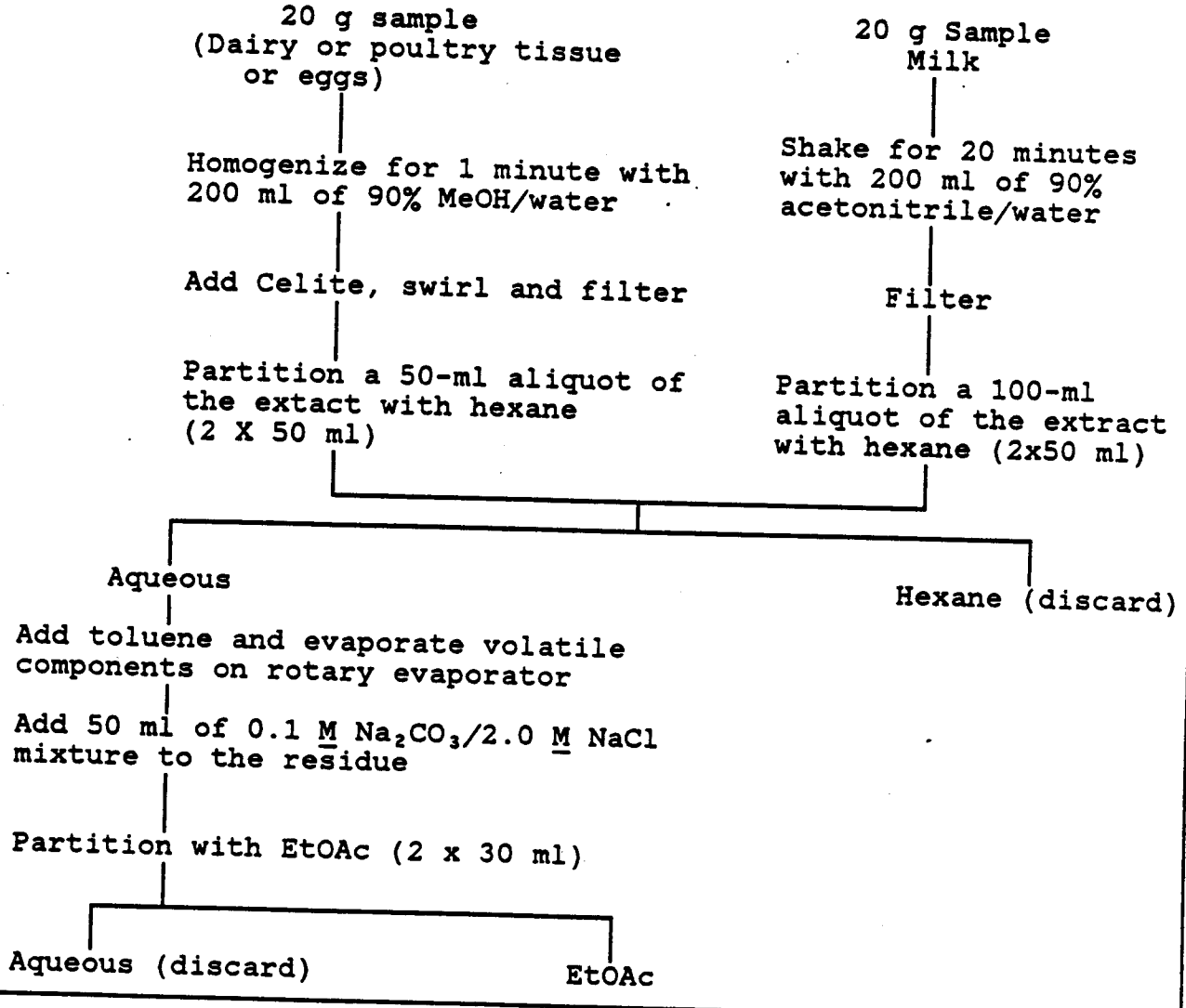
Substrate	0.01 ppm	0.05 ppm	0.10 ppm	0.50 ppm
Dairy Milk	84,84,89,97,98	95	90,97,103,103	95,96,99

No apparent residues (<0.01 ppm) were observed in the unfortified samples.

9.2 No difference in appearance of HPLC chromatograms was observed using acetonitrile:water (9:1) instead of methanol:water (9:1) for extraction of milk.

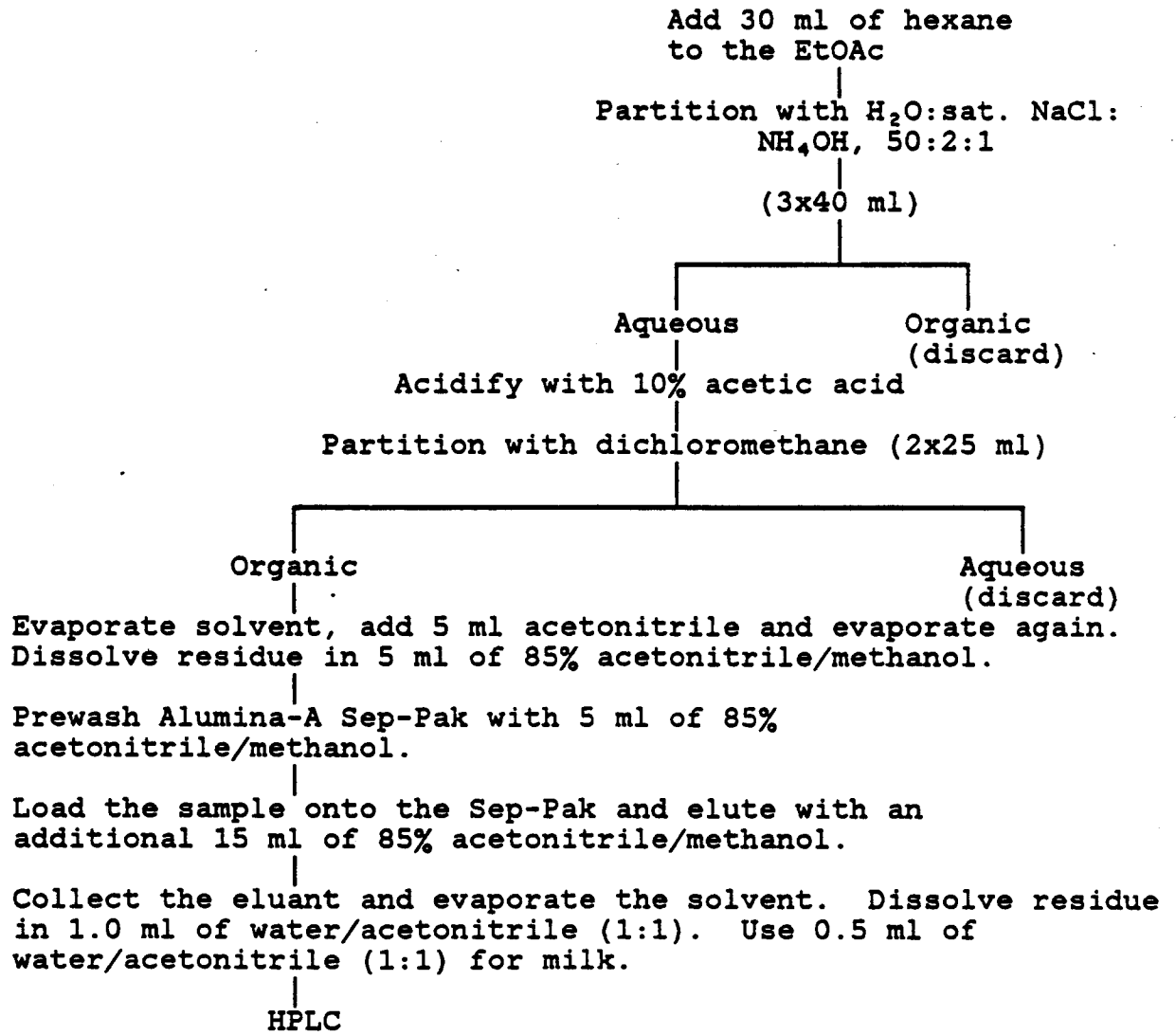
PAGE 3 of 5	METHOD NO. AG-506A	SUBJECT
EDITION		ADDENDUM TO AG-506: SUBSTITUTION OF ACETONITRILE FOR METHANOL FOR EXTRACTION OF CGA-136872 RESIDUES FROM MILK
SUBMITTED BY: K. Van Geluwe-Barvir, T. Beidler		
		APPROVED BY:

FIGURE 1: FLOW DIAGRAM FOR ANALYTICAL METHOD FOR DETERMINATION OF CGA-136872 IN DAIRY AND POULTRY TISSUES, EGGS, AND MILK BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY



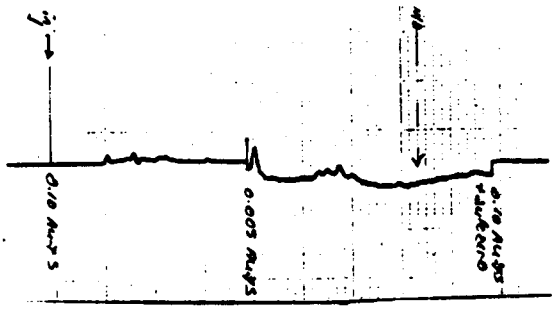
PAGE 4 of 5	METHOD NO. AG-506A	SUBJECT
EDITION		ADDENDUM TO AG-506: SUBSTITUTION OF ACETONITRILE FOR METHANOL FOR EXTRACTION OF CGA-136872 RESIDUES FROM MILK
SUBMITTED BY: K. Van Geluwe-Barvir, T. Beidler		
		APPROVED BY:

FIGURE 1: FLOW DIAGRAM FOR ANALYTICAL METHOD FOR DETERMINATION OF CGA-136872 IN DAIRY AND POULTRY TISSUES, EGGS, AND MILK BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (Continued)

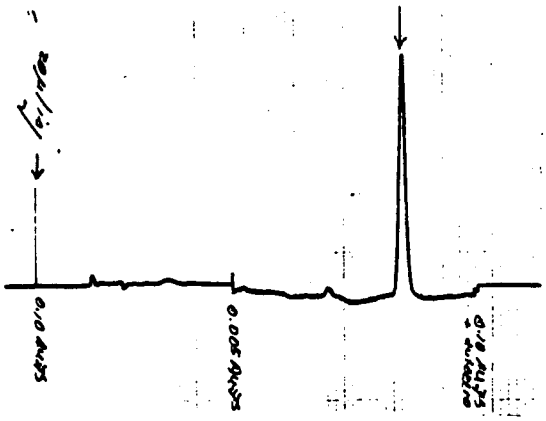


PAGE 5 of 5	METHOD NO. AG-506A	SUBJECT
EDITION		ADDENDUM TO AG-506: SUBSTITUTION OF ACETONITRILE FOR METHANOL FOR EXTRACTION OF CGA-136872 RESIDUES FROM MILK
SUBMITTED BY: K. Van Geluwe-Barvir, T. Beidler		
		APPROVED BY:

FIGURE 2: TYPICAL CHROMATOGRAMS FOR MILK SAMPLES (AG-A 10242)



Control Milk
368 mg injected
<2 ng CGA-136872 found
<0.01 ppm



Control + 0.10 ppm
92 mg injected
8.9 ng CGA-136872 found
97% recovery

Treated sample
4-7-A
368 mg injected
8.5 ng found
0.02 PPM

CERTIFICATION

The reports included in this study, Laboratory Project I.D. AG-506, are certified to be authentic accounts of the experiments, and the results of these experiments, described herein.

8/13/87
Date

Robert K. Williams
Research Scientist/Group Leader
Analytical Method Development Group
New Product Chemistry
Biochemistry Department
919-292-7100, Ext. 2295

AGRICULTURAL DIVISION
CIBA-GEIGY CORPORATION
POST OFFICE BOX 18300
GREENSBORO, NC 27419

CIBA-GEIGY Corporation AG-A 9870-01 Page 1 of 3
Field Test Number M6-161-5A Project Number 161949

Compound(s) and Formulations(s): Ø-^{14}C-CGA-136872 99% Chemical Purity (CL-IV-46)		Commodity: Goat	Substrate: Liver
C-G Rep.:	Plot Location:	Growth Stages Sampled:	Cooperator Name and Address:
Soil Type:	Date Planted:		

Treatment Rates:	Method of Application:	Equipment:	Vol. per Acre:

Dates of Application:	Sampling Date(s):
Other Materials Applied:	Sample Care Before Storage:

Storage Information:	No. of Analyses: 6	Plot Maintenance, i.e., Cultivation, Irrigation, etc.:

Summary of Results: At the CIBA-GEIGY Research Facility in Vero Beach, Florida, a lactating goat was treated with eleven daily consecutive oral doses of Ø-^{14}C-CGA-136872 (31.7 µCi/mg) at a level equivalent to approximately 4 ppm in the feed. Twenty-three hours after the last dose, the goat was sacrificed and samples of the tissues were collected. Liver from this goat was analyzed for parent CGA-136872 residues by AG-506 and the results are shown below along with the percent of total radioactive residue present in the goat liver extract and the final fraction (used for HPLC analysis) as determined by liquid scintillation counting of aliquots from these solutions.

Test No.	Sample	Total ppm ^{14}C	% ^{14}C in Extract	% ^{14}C (ppm) in Final Fraction	HPLC Results (ppm) Uncorrected for Procedural Recovery	HPLC Results (ppm) Corrected for Procedural Recovery
M6-161-5A*	#36-Liver B	0.120	87%	17% (0.021)	<0.05 (0.02)	<0.05 (0.03)

*See ABR-85076 for details.
Distribution: R. A. Kahrs, J. A. Ross, R. K. Williams, Main File

Date Received:	Date Extracted: 9/22/86	Date Analyzed: 11/4/86	Analyst: WTB
Method of Analysis: AG-506			
Analysis Approved By:			Date Approved: 1/15/87

December/dg/A

CIBA-GEIGY Corporation

Compound(s) Ø-^{14}C-CGA-136872					Substrate Goat Liver						
						Residue - ppm					
Sample Code	ppm	Formulation Spray Additives Other Treatment Data	Application Date(s)	Sample Date(s)	Interval (Days)	Ø-^{14}C-CGA-136872					
Goat #55 (Liver B)	0.00	--	--	6/4/85	--	<0.05					
Goat #36 (Liver B)	4.00*	--	5/24-6/4/85	6/4/85	--	<0.05(0.03)					

Ø-^{14}C-CGA-136872: methyl 2-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl][U-ring-^{14}C]benzoate

Comments: Residue results are not corrected for control values. Residue results are corrected for procedural recoveries of <100% and for moisture content of 80% by formula in Section 6.3.2 of AG-506.
*Average daily dose based on the feed.

December/dg/A (rev. 1/13/86 kf)

PG0046 00076

RESIDUE RECOVERY REPORT

AG-A No. 9870-01

Compound(s) CGA-136872		Substrate(s) Goat Liver					
		Pesticide Added CGA-136872					
SUBSTRATE	SAMPLE WEIGHT	AMOUNT ADDED		FOUND			
				TOTAL		NET	
	mg	ng	ppm	ng	ppm*	ppm	%
Control Liver, Goat #55-Liver B	100	0.0	0.00	<2.0	<0.05	<0.05	
CGA-136872	100	5.0	0.05	4.4	0.047	0.047	94
CGA-136872	100	5.0	0.05	3.9	0.042	0.042	84
CGA-136872	100	10.0	0.10	7.9	0.085	0.085	85
CGA-136872	100	20.0	0.20	16.4	0.178	0.178	89

Comments: Recovery samples fortified prior to extraction.
*Liver ppm values are corrected for 80% moisture content by formula in Section 6.4.2 of AG-506.

December/dg/A

CIBA-GEIGY Corporation

AG-A 9871-01

Field Test Number

Project Number 161949

Compound(s) and Formulations(s): CGA-136872 (Analytical Standard, S85-0813)		Commodity:	Substrate: Meat, Milk, Poultry and Eggs (Method Trials)
C-G Rep.:	Plot Location:	Growth Stages Sampled:	Cooperator Name and Address:
Soil Type:	Date Planted:		

Treatment Rates:	Method of Application:	Equipment:	Vol. per Acre:

Dates of Application:	Sampling Date(s):
Other Materials Applied:	Sample Care Before Storage:

Storage Information:	No. of Analyses: 70	Plot Maintenance, i.e., Cultivation, Irrigation, etc.:

Summary of Results: See Page 2 for summary of recovery data.

Distribution: R. A. Kahrs
J. A. Ross
R. K. Williams
M. File

CGA-136872: methyl 2-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoate

Date Received:	Date Extracted: 10/86 - 12/86	Date Analyzed: 10/86 - 12/86	Analyst: WTB

Method of Analysis: AG-506

Analysis Approved By:	Date Approved: 1/16/87

DEC./1a/A

Summary of Results: Method trials for validation of Analytical Method AG-506 were conducted on dairy blood and tissue, poultry tissue, egg and milk control samples obtained from CIBA-GEIGY residue studies. Control and fortified control samples were analyzed with the following results:

Test No.	Substrate	Sample	Control (ppm)	Fortification Level (ppm)	Average Recovery (%)
BC-IR-002-83	Whole Milk	12M-A	<0.01	0.01 - 0.20	90
BC-IR-002-83	Blood	1-19-A	<0.05	0.05 - 0.20	90
BC-IR-002-83	Dairy Loin	1-42-A	<0.05	0.05 - 0.20	86
BC-FR-003-84	Dairy Round	1-20-B	<0.05	0.05 - 0.20	94
BC-IR-002-83	Dairy Liver	1-20-B	<0.05	0.05 - 1.00	92
BC-IR-002-83	Dairy Perirenal Fat	1-39-B	<0.05	0.05 - 0.20	95
BC-IR-002-83	Dairy Omental Fat	1-40-B	<0.05	0.05 - 0.20	98
BC-IR-002-83	Dairy Kidney	1-43-B	<0.05	0.05 - 1.00	91
BC-FR-004-84	Chicken Lean Meat	1-23-A	<0.05	0.05 - 0.20	91
BC-FR-004-84	Eggs	1-10-A	<0.05	0.05 - 0.20	86
BC-FR-004-84	Chicken Skin	1-20-A	<0.05	0.05 - 0.20	85
BC-FR-004-84	Chicken Liver	1-30-A	<0.05	0.05 - 1.00	90
BC-FR-004-84	Chicken Fat	1-29-A	<0.05	0.05 - 0.20	92

DEC./la/A

AG-A No. 9871-01

Compound(s) CGA-136872		Substrate(s) Control Whole Milk, Dairy Blood and Loin					
		Pesticide Added CGA-136872					
SUBSTRATE	SAMPLE WEIGHT mg	AMOUNT ADDED		FOUND			
				TOTAL		NET	
		ng	ppm	ng	ppm*	ppm	%
Control Whole Milk (12M-A)	400.	0.0	0.00	<2.0	<0.01	<0.01	
CGA-136872	400.	4.0	0.01	3.3	0.009	0.009	91
CGA-136872	400.	4.0	0.01	3.5	0.010	0.010	96
CGA-136872	400.	4.0	0.01	2.8	0.008	0.008	75
CGA-136872	200.	20.	0.10	16.8	0.092	0.092	92
CGA-136872	100.	20.	0.20	17.5	0.190	0.190	95
Control Blood (1-19-A)	100.	0.0	0.00	<2.0	<0.05	<0.05	
CGA-136872	100.	5.0	0.05	4.1	0.044	0.044	88
CGA-136872	100.	5.0	0.05	4.4	0.048	0.048	96
CGA-136872	100.	10.0	0.10	8.0	0.086	0.086	86
CGA-136872	100.	20.	0.20	16.5	0.179	0.179	89
Control Dairy Loin (1-42-A)	100.	0.0	0.00	<2.0	<0.05	<0.05	
CGA-136872	100.	5.0	0.05	3.9	0.042	0.042	84
CGA-136872	100.	5.0	0.05	3.7	0.040	0.040	80
CGA-136872	100.	10.0	0.10	7.5	0.080	0.080	80
CGA-136872	100.	20.	0.20	18.2	0.196	0.196	98

Comments: Recovery samples fortified prior to extraction.

*Milk ppm values are corrected for 87% moisture content, and blood and loin ppm values are corrected for 80% moisture content using formula in Section 6.4.2 of AG-506.

DEC./la/A

AG-A No. 9871-01

Compound(s) CGA-136872	Substrate(s) Control Dairy Roundmeat, Liver, and Perirenal Fat Pesticide Added CGA-136872						
SUBSTRATE	SAMPLE WEIGHT	AMOUNT ADDED		FOUND			
				TOTAL		NET	
	mg	ng	ppm	ng	ppm*	ppm	%
Control Dairy Roundmeat (1-20-B)	100.	0.0	0.00	<2.0	<0.05	<0.05	
CGA-136872	100.	5.0	0.05	4.9	0.053	0.053	106
CGA-136872	100.	5.0	0.05	4.5	0.049	0.049	97
CGA-136872	100.	10.0	0.10	7.9	0.085	0.085	85
CGA-136872	100.	20.	0.20	16.2	0.175	0.175	87
Control Dairy Liver (1-20-B)	100.	0.0	0.00	<2.0	<0.05	<0.05	
CGA-136872	100.	5.0	0.05	4.5	0.048	0.048	96
CGA-136872	100.	5.0	0.05	4.2	0.046	0.046	92
CGA-136872	100.	10.0	0.10	8.4	0.091	0.091	91
CGA-136872	100.	20.	0.20	17.3	0.187	0.187	93
CGA-136872	20.	20.	1.00	16.2	0.88	0.88	88
Control Dairy Perirenal Fat (1-39-B)	100.	0.0	0.00	<2.0	<0.05	<0.05	
CGA-136872	100.	5.0	0.05	4.5	0.045	0.045	91
CGA-136872	100.	5.0	0.05	5.0	0.050	0.050	100
CGA-136872	100.	10.0	0.10	9.4	0.094	0.094	94
CGA-136872	100.	20.	0.20	18.9	0.189	0.189	95

Comments: Recovery samples fortified prior to extraction.

*Dairy roundmeat and liver ppm values are corrected for 80% moisture content by formula in Section 6.4.2 of AG-506.

DEC./la/A

AG-A No. 9871-01

Compound(s) CGA-136872		Substrate(s) Control Dairy Omental Fat and Kidney and Chicken Lean Meat					
		Pesticide Added CGA-136872					
SUBSTRATE	SAMPLE WEIGHT mg	AMOUNT ADDED		FOUND			
		ng	ppm	TOTAL ng	TOTAL ppm*	NET ppm	NET %
Control Dairy Omental Fat (1-40-B)	100.	0.0	0.00	<2.0	<0.05	<0.05	
CGA-136872	100.	5.0	0.05	5.5	0.055	0.055	110
CGA-136872	100.	5.0	0.05	4.5	0.045	0.045	89
CGA-136872	100.	10.0	0.10	9.5	0.095	0.095	95
CGA-136872	100.	20.	0.20	19.4	0.194	0.194	97
Control Dairy Kidney (1-43-B)	100.	0.0	0.00	<2.0	<0.05	<0.05	
CGA-136872	100.	5.0	0.05	4.3	0.046	0.046	92
CGA-136872	100.	5.0	0.05	4.0	0.044	0.044	87
CGA-136872	100.	10.0	0.10	8.7	0.094	0.094	94
CGA-136872	100.	20.	0.20	17.4	0.188	0.188	94
CGA-136872	20.	20.	1.00	16.5	0.89	0.89	89
Control Chicken Lean Meat (1-23-A)	100.	0.0	0.00	<2.0	<0.05	<0.05	
CGA-136872	100.	5.0	0.05	4.1	0.044	0.044	89
CGA-136872	100.	5.0	0.05	4.4	0.047	0.047	94
CGA-136872	100.	10.0	0.10	8.5	0.092	0.092	92
CGA-136872	100.	20.	0.20	16.4	0.177	0.177	89

Comments: Recovery samples fortified prior to extraction.

*Dairy kidney and chicken lean meat ppm values are corrected for 80% moisture content by formula in Section 6.4.2 of AG-506.

DEC./1a/A

AG-A No. 9871-01

Compound(s) CGA-136872		Substrate(s) Control Chicken Eggs, Skin and Liver					
		Pesticide Added CGA-136872					
SUBSTRATE	SAMPLE WEIGHT mg	AMOUNT ADDED		FOUND			
				TOTAL		NET	
		ng	ppm	ng	ppm*	ppm	%
Control Eggs (1-10-A)	100.	0.0	0.00	<2.0	<0.05	<0.05	
CGA-136872	100.	5.0	0.05	4.2	0.045	0.045	90
CGA-136872	100.	5.0	0.05	3.7	0.040	0.040	79
CGA-136872	100.	10.0	0.10	8.1	0.086	0.086	87
CGA-136872	100.	20.	0.20	16.5	0.177	0.177	89
Control Chicken Skin (1-20-A)	100.	0.0	0.00	<2.0	<0.05	<0.05	
CGA-136872	100.	5.0	0.05	4.7	0.047	0.047	95
CGA-136872	100.	5.0	0.05	3.9	0.039	0.039	78
CGA-136872	100.	10.0	0.10	8.1	0.081	0.081	81
CGA-136872	100.	20.	0.20	17.1	0.171	0.171	86
Control Chicken Liver (1-30-A)	100.	0.0	0.00	<2.0	<0.05	<0.05	
CGA-136872	100.	5.0	0.05	4.2	0.046	0.046	91
CGA-136872	100.	5.0	0.05	3.8	0.041	0.041	82
CGA-136872	100.	10.0	0.10	8.7	0.094	0.094	94
CGA-136872	100.	20.	0.20	16.9	0.182	0.182	91
CGA-136872	20.	20.	1.00	17.2	0.93	0.93	93

Comments: Recovery samples fortified prior to extraction.

*Chicken liver ppm values are corrected for 80% moisture content and egg ppm values are corrected for 74% moisture content by formula in Section 6.4.2 of AG-506.

DEC./la/A

RESIDUE RECOVERY REPORT

AG-A No. 9871-01

Compound(s) CGA-136872		Substrate(s) Control Chicken Fat					
		Pesticide Added CGA-136872					
SUBSTRATE	SAMPLE WEIGHT	AMOUNT ADDED		FOUND			
				TOTAL		NET	
	mg	ng	ppm	ng	ppm	ppm	%
Control Chicken Fat (1-29-A)	100.	0.0	0.00	<2.0	<0.05	<0.05	
CGA-136872	100.	5.0	0.05	4.2	0.042	0.042	84
CGA-136872	100.	5.0	0.05	4.6	0.046	0.046	93
CGA-136872	100.	10.0	0.10	9.7	0.097	0.097	97
CGA-136872	100.	20.	0.20	18.6	0.186	0.186	93

Comments: Recovery samples fortified prior to extraction.

DEC./1a/A

BIOCHEMISTRY DEPARTMENT
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GREENSBORO, NC

VALIDATION OF ANALYTICAL METHOD AG-506 FOR THE DETERMINATION OF CGA-136872 IN DAIRY AND POULTRY TISSUES, EGGS AND MILK BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Report No.: ABR-87076

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I. INTRODUCTION AND SUMMARY

CGA-136872, methyl 2-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoate is an experimental herbicide being developed by CIBA-GEIGY for use in corn. Analytical Method AG-506[1] has been developed for the determination of residues of CGA-136872 in poultry tissues (lean meat, skin plus adhering fat, liver and fat), eggs, dairy cow blood and tissues (round, loin, kidney, liver, perirenal fat and omental fat) and whole milk. The detection limit for the method is 0.01 ppm of CGA-136872 in milk and 0.05 ppm in the other substrates. The validity of the method was tested by analysis of control and fortified control samples from CIBA-GEIGY residue studies and by analysis of liver from a goat dosed with radiolabelled CGA-136872.

Untreated meat, milk and egg samples were fortified in the range of 0.01 ppm to 1.00 ppm of CGA-136872 and were analyzed by AG-506. Residues of CGA-136872, either real or apparent, were less than 0.05 ppm (less than 0.01 ppm in milk) in all control samples. Recovery values averaged 91% with a standard deviation of 8.1% (n=27) at the screening level of 0.05 ppm (0.01 ppm in milk) and 91% with a standard deviation of 6.5% (n=56) overall.

Analysis of liver from a ϕ-^{14}C-CGA-136872-dosed goat by AG-506 accounted for 21% of the total radioactive residues as CGA-136872 after correcting for procedural recoveries. Extractability of the total radioactive residue from the liver was 87%. These results indicate that Analytical Method AG-506 is valid for the determination of CGA-136872 in meat, milk and eggs.

Work on this study was begun on September 22, 1986 and was completed on December 8, 1986.

II. MATERIALS

A. Test Substances

Procedural recoveries were determined from control samples which were fortified with standard CGA-136872 (S-850813, purity not established), methyl 2-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]

carbonyl]amino]sulfonyl]benzoate, dissolved in acetonitrile/water (1:1). The goat liver from Metabolism Study M6-161-5A[2] was taken from a goat treated with ϕ-^{14}C-CGA-136872 (31.7 μCi/mg, 99% radiochemical purity, CL-IV-46).

B. Test Commodity

Control samples of poultry and dairy tissue, milk and eggs were selected from a number of CIBA-GEIGY residue studies. Details on these samples can be found in Table I.

Radioactive goat liver was obtained from Metabolism Study M6-161-5A[2]. The biological portion[3] of this study was conducted at the CIBA-GEIGY Research Facility in Vero Beach, Florida where a lactating goat was treated with eleven consecutive daily oral doses of ϕ-^{14}C-CGA-136872 at a level equivalent to 4 ppm in the feed. Twenty-three hours after the last dose the goat was sacrificed and tissue samples were collected.

III. METHOD

A. Experimental Design

As part of the validation of analytical method AG-506 for use in the determination of parent CGA-136872 residues in meat, milk and eggs obtained from three-level dairy and poultry studies, a number of analyses were performed on control samples from other CIBA-GEIGY animal residue studies. For each substrate a control sample along with duplicate controls fortified with CGA-136872 at the screening level of 0.05 ppm (triplicate controls at 0.01 ppm in milk) were analyzed by AG-506. Two more control samples were fortified at 0.10 and 0.20 ppm for every substrate and an additional 1.00 ppm fortification was run with liver and kidney where higher residues were expected.

The second part of method validation involved the analysis of a liver sample from a goat dosed with radiolabelled CGA-136872. In this way extractability of the radioactive residue from animal tissue and accountability for parent compound in the total residue could be demonstrated. The treated sample was analyzed by AG-506 simultaneously with a control and controls fortified at 0.05 ppm (duplicate analysis), 0.10 ppm and 0.20 ppm with CGA-136872. Total radioactivity in the tissue extract and in the final fraction from analysis of the treated sample was determined by liquid scintillation counting of aliquots from the extract and the final fraction. The radioactivity value from the final fraction was then compared with the CGA-136872 residue found by HPLC analysis of the final fraction.

B. Analytical Method AG-506

Parent residues of CGA-136872 were extracted from dairy and poultry tissues, eggs and milk by homogenizing weighed samples in 90% methanol/water for one minute using a Polytron homogenizer. The extract was filtered after addition of diatomaceous earth, then an aliquot was removed and partitioned with hexane. The methanol/water layer was evaporated to a small volume, diluted with a solution of sodium carbonate (0.1 M) and sodium chloride (2.0 M), then partitioned with ethyl acetate. After adding hexane, the ethyl acetate was partitioned several times with water/saturated sodium chloride/concentrated ammonium hydroxide, 50:2:1. The aqueous layers were combined, acidified with 10% acetic acid and partitioned with dichloromethane. The dichloromethane was evaporated, acetonitrile was added and the evaporation process repeated to remove any residual water. Final cleanup was performed with an Alumina-A Sep-Pak. Residues of CGA-136872, in all substrates except milk, were determined by HPLC on a Zorbax-ODS column using a mobile phase comprised of 56% acetonitrile and 44% phosphate buffer with UV detection at 234 nm. For the determination of CGA-136872 residues in milk, a mobile phase comprised of 54% acetonitrile and 46% phosphate buffer was used.

IV. RESULTS AND DISCUSSION

The recovery data for various substrates analyzed by AG-506 are shown in Table I and are reported in AG-A 9871-01. The data show an average recovery of 91% (S.D.=8.1%, n=27) at the screening level of 0.05 ppm (0.01 ppm in milk) and 91% (S.D.=6.5%, n=56) overall. Backgrounds in control samples were below the screening level in all substrates. Chromatograms from each substrate are shown in Figures 1 to 13.

The results for the analysis by AG-506 of ϕ-^{14}C-CGA-136872 treated goat liver are shown in Table II and are reported in AG-A 9870-01. Of the total radioactive residue, 87% was extractable into 90% MeOH/water. Upon analysis of the extract by the procedures of AG-506, 17% of the total radioactive residue was found in the final fraction. Analysis of the final fraction by HPLC gave less than 0.05 ppm of CGA-136872. Actual values approximated from the HPLC run were 0.02 ppm of CGA-136872 (18% of the total radioactivity) uncorrected and 0.03 ppm of CGA-136872 (21% of the total radioactivity) after correcting for procedural recovery determined from the CGA-136872 fortified samples. In the goat metabolism study[2], the contribution from parent CGA-136872 to the total radioactive residue (0.083 ppm) was reported as 33%.

V. CONCLUSIONS

Analytical method AG-506 is valid for the determination of parent residues of CGA-136872 in poultry tissues (lean meat, skin plus adhering fat, liver and fat), eggs, dairy cow blood and tissues (round, loin, kidney, liver, perirenal fat and omental fat) and whole milk. Validation was accomplished by analyzing control, fortified control and ϕ-^{14}C-CGA-136872 treated samples. Background levels were less than the screening level of 0.05 ppm (0.01 ppm in milk) in all control samples. The average recovery of CGA-136872 from fortified control samples was 91% (S.D.=6.5%, n=56) overall and 91% (S.D.=8.1, n=27) at the screening level. Analysis of liver from a ϕ-^{14}C-CGA-136872-dosed goat by AG-506 accounted for 21% of the total radioactive residue as CGA-136872. Extractability of the total radioactive residue from the liver of a goat treated eleven consecutive days with 4 ppm of ϕ-^{14}C-CGA-136872 was 87%.

VI. TABLES AND FIGURES

TABLE I: SUMMARY OF RECOVERY DATA FOR MEAT, MILK AND EGG SAMPLES FORTIFIED WITH CGA-136872 (AG-A 9871-01)

Substrates	Test No.	Sample Code	% Recoveries at Various Fortification Levels 0.01 ppm	0.05 ppm	0.10 ppm	0.20 ppm	1.00 ppm
Milk	BC-IR-002-83	12M-A	91,96, 75		92	95	
Dairy Blood	BC-IR-002-83	1-19-A		86,96	86	89	
Dairy Loin	BC-IR-002-83	1-42-A		84,80	80	98	
Dairy Round	BC-FR-003-84	1-20-B		106,97	85	87	
Dairy Perirenal Fat	BC-IR-002-83	1-39-B		91,100	94	95	
Dairy Omental Fat	BC-IR-002-83	1-40-B		110,89	95	97	
Dairy Liver	BC-IR-002-83	1-20-B		96,92	91	93	88
Dairy Kidney	BC-IR-002-83	1-43-B		92,87	94	94	89
Chicken Lean Meat	BC-FR-004-84	1-23-A		89,94	92	89	
Chicken Skin Plus Adhering Fat	BC-FR-004-84	1-20-A		95,78	81	86	
Chicken Fat	BC-FR-004-84	1-29-A		84,93	97	93	
Chicken Liver	BC-FR-004-84	1-30-A		91,82	94	91	93
Eggs	BC-FR-004-84	1-10-A		90,79	87	89	

Average recovery at the screening level of 0.05 ppm (0.01 ppm in milk) is 91% (S.D. = 8.1%, n=27).

Average recovery for all levels is 91% (S.D. = 6.5%, n=56).

No residues at or above the screening level of 0.05 ppm (0.01 ppm in milk) were found in any of the control samples.

TABLE II: ANALYSIS OF LIVER FROM A GOAT DOSED WITH ϕ-^{14}C-CGA-136872[a]

AGA No.: 9870-01

Test No.: M6-161-5A

Location: CIBA-GEIGY Research Facility at Vero Beach, Florida

Sample: Goat #36, Liver(Rep B)

Total ppm[b]: 0.120

Results of analysis by AG-506

Percent of total ^{14}C in extract[c]: 87%

Percent (ppm) of total ^{14}C in final fraction[c]: 17% (0.021)

CGA-136872 in final fraction determined by HPLC:

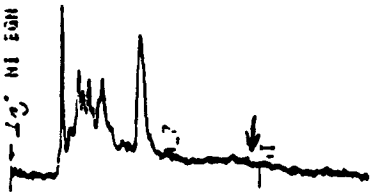
Uncorrected for procedural recovery	<0.05 ppm (0.02 ppm or 18% of the total radioactive residue)
Corrected for procedural recovery of 88%	<0.05 ppm (0.03 ppm or 21% of the total radioactive residue)

a) One lactating goat was treated with eleven consecutive daily oral doses of ϕ-^{14}C-CGA-136872 at a level equivalent to approximately 4 ppm in the feed. Twenty-three hours after the last dose, the goat was sacrificed and samples of the tissues were collected. See reference 2 for details.

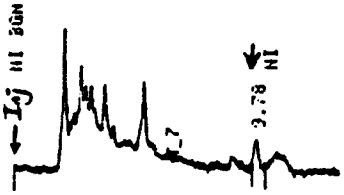
b) Total ppm determined by combustion and measurement of CO_2 in accordance with SOP No. 4.67[4].

c) Determined by liquid scintillation counting of aliquots of solutions[5].

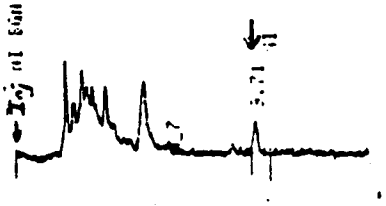
FIGURE 1: TYPICAL CHROMATOGRAMS FOR MILK



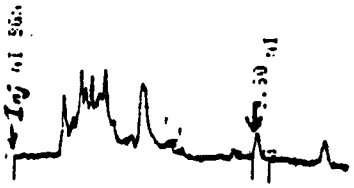
Control Milk
368 mg injected
<2 ng CGA-136872
<0.01 ppm



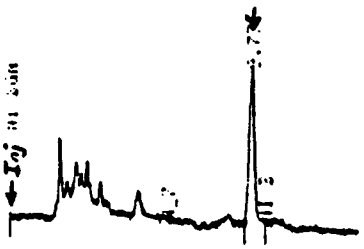
Control + 0.01 ppm
368 mg injected
3.33 ng CGA-136872
91% recovery



Control + 0.01 ppm
368 mg injected
3.53 ng CGA-136872
96% recovery



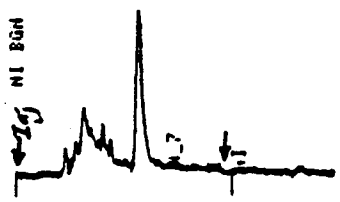
Control + 0.01 ppm
368 mg injected
2.75 ng CGA-136872
75% recovery



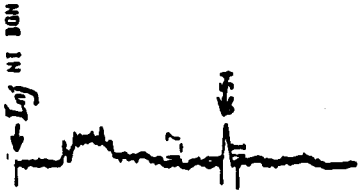
Control + 0.10 ppm
184 mg injected
16.8 ng CGA-136872
92% recovery

Control 0.20 ppm
92 mg injected
17.5 ng CGA-136872
95% recovery

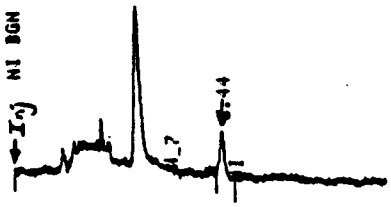
FIGURE 2: TYPICAL CHROMATOGRAMS FOR DAIRY BLOOD



Control Blood
92.6 mg injected
<2 ng CGA-136872
<0.05 ppm

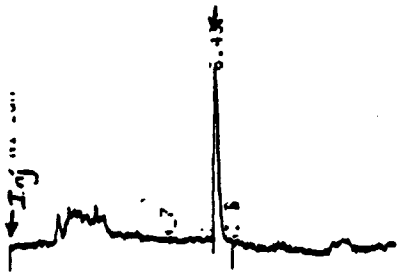


Control + 0.05 ppm
92.6 mg injected
4.10 ng CGA-136872
88% recovery



Control + 0.05 ppm
92.6 mg injected
4.42 ng CGA-136872
96% recovery

Control + 0.10 ppm
92.6 mg injected
8.00 ng CGA-136872
86% recovery



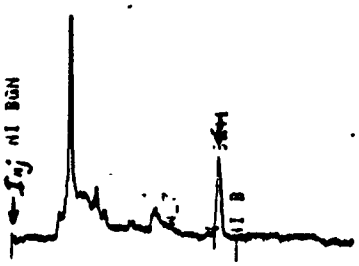
Control + 0.20 ppm
92.6 mg injected
16.5 ng CGA-136872
89% recovery

FIGURE 3: TYPICAL CHROMATOGRAMS FOR DAIRY LOIN MEAT

Control Loin
92.6 mg injected
<2 ng CGA-136872
<0.05 ppm

Control + 0.05 ppm
92.6 mg injected
3.90 ng CGA-136872
84% recovery

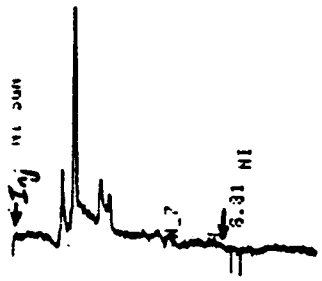
Control + 0.05 ppm
92.6 mg injected
3.72 ng CGA-136872
80% recovery



Control + 0.10 ppm
92.6 mg injected
7.45 ng CGA-136872
80% recovery

Control + 0.20 ppm
92.6 mg injected
18.2 ng CGA-136872
98% recovery

FIGURE 4: TYPICAL CHROMATOGRAMS FOR DAIRY ROUND MEAT

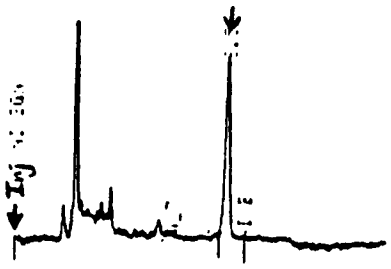


Control Round
92.6 mg injected
<2 ng CGA-136872
<0.05 ppm

Control + 0.05 ppm
92.6 mg injected
4.91 ng CGA-136872
106% recovery

Control + 0.05 ppm
92.6 mg injected
4.51 ng CGA-136872
97% recovery

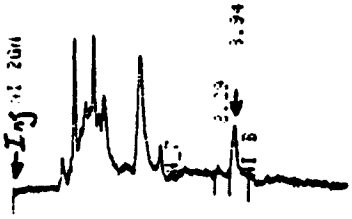
Control + 0.10 ppm
92.6 mg injected
7.90 ng CGA-136872
85% recovery



Control + 0.20 ppm
92.6 mg injected
16.2 ng CGA-136872
87% recovery

FIGURE 5: TYPICAL CHROMATOGRAMS FOR DAIRY LIVER

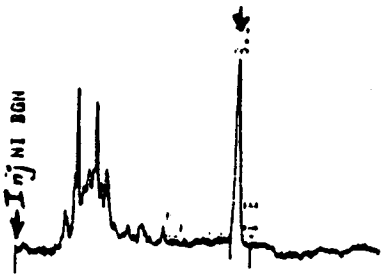
Control Liver
92.6 mg injected
<2 ng CGA-136872
<0.05 ppm



Control + 0.05 ppm
92.6 mg injected
4.46 ng CGA-136872
96% recovery

Control + 0.05 ppm
92.6 mg injected
4.25 ng CGA-136872
92% recovery

Control + 0.10 ppm
92.6 mg injected
8.43 ng CGA-136872
91% recovery



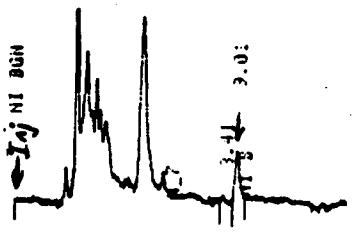
Control + 0.20 ppm
92.6 mg injected
17.3 ng CGA-136872
93% recovery

Control + 1.00 ppm
18.5 mg injected
16.2 ng CGA-136872
88% recovery

FIGURE 6: TYPICAL CHROMATOGRAMS FOR DAIRY KIDNEY

Control Kidney
92.6 mg injected
<2 ng CGA-136872
<0.05 ppm

Control + 0.05 ppm
92.6 mg injected
4.28 ng CGA-136872
92% recovery

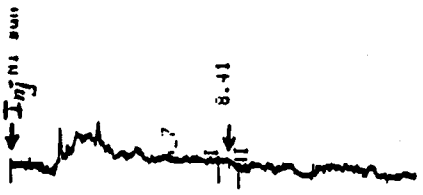


Control + 0.05 ppm
92.6 mg injected
4.03 ng CGA-136872
87% recovery

Control + 0.10 ppm
92.6 mg injected
8.71 ng CGA-136872
94% recovery

Control + 0.20 ppm
92.6 mg injected
17.4 ng CGA-136872
94% recovery

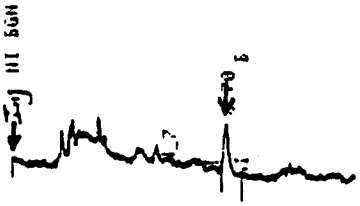
Control + 1.00 ppm
18.5 mg injected
16.5 ng CGA-136872
89% recovery



Reagent Blank
92.6 mg injected
<2 ng CGA-136872
<0.05 ppm

FIGURE 7: TYPICAL CHROMATOGRAMS FOR DAIRY PERIRENAL FAT

Control Fat
100 mg injected
<2 ng CGA-136872
<0.05 ppm



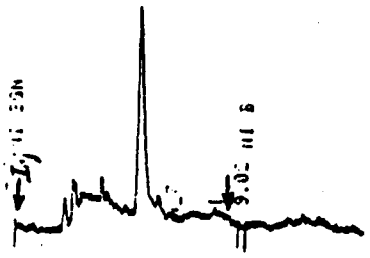
Control + 0.05 ppm
100 mg injected
4.54 ng CGA-136872
91% recovery

Control + 0.05 ppm
100 mg injected
4.99 ng CGA-136872
100% recovery

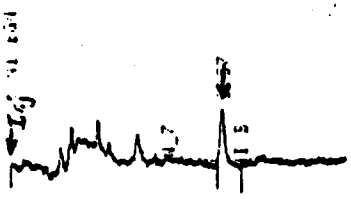
Control + 0.10 ppm
100 mg injected
9.37 ng CGA-136872
94% recovery

Control + 0.20 ppm
100 mg injected
18.9 ng CGA-136872
95% recovery

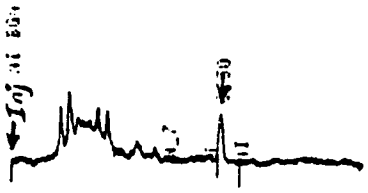
FIGURE 8: TYPICAL CHROMATOGRAMS FOR DAIRY OMENTAL FAT



Control Fat
100 mg injected
<2 ng CGA-136872
<0.05 ppm



Control + 0.05 ppm
100 mg injected
5.49 ng CGA-136872
110% recovery



Control + 0.05 ppm
100 mg injected
4.47 ng CGA-136872
89% recovery

Control + 0.10 ppm
100 mg injected
9.51 ng CGA-136872
95% recovery

Control + 0.20 ppm
100 mg injected
19.4 ng CGA-136872
97% recovery

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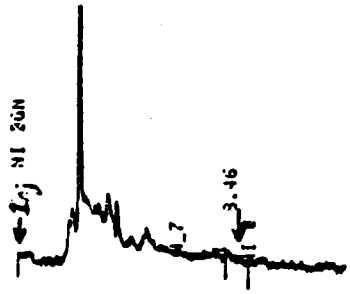
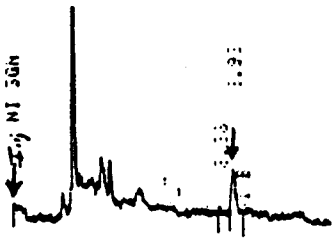
FIGURE 9: TYPICAL CHROMATOGRAMS FOR CHICKEN LEAN MEAT



Control Chicken Meat
92.6 mg injected
<2 ng CGA-136872
<0.05 ppm

Control + 0.05 ppm
92.6 mg injected
4.11 ng CGA-136872
89% recovery



Control + 0.05 ppm
92.6 mg injected
4.35 ng CGA-136872
94% recovery

Control + 0.10 ppm
92.6 mg injected
8.54 ng CGA-136872
92% recovery

Control + 0.20 ppm
92.6 mg injected
16.4 ng CGA-136872
89% recovery

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FIGURE 10: TYPICAL CHROMATOGRAMS FOR CHICKEN LIVER

Control Liver
92.6 mg injected
<2 ng CGA-136872
<0.05 ppm

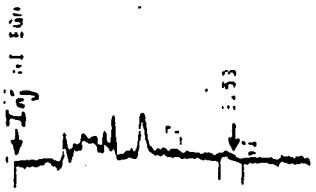
Control + 0.05 ppm
92.6 mg injected
4.23 ng CGA-136872
91% recovery

Control + 0.05 ppm
92.6 mg injected
3.80 ng CGA-136872
82% recovery

Control + 0.10 ppm
92.6 mg injected
8.67 ng CGA-136872
94% recovery

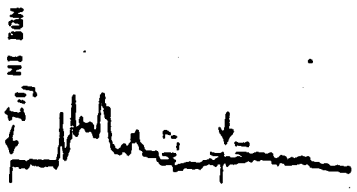
Control + 0.20 ppm
92.6 mg injected
16.9 ng CGA-136872
91% recovery

Control + 1.00 ppm
18.5 mg injected
17.2 ng CGA-136872
93% recovery

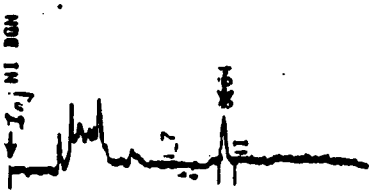


Reagent Blank
92.6 mg injected
<2 ng CGA-136872
<0.05 ppm

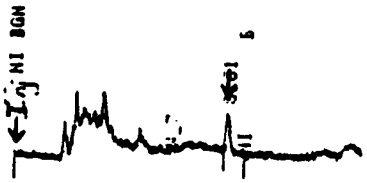
FIGURE 11: TYPICAL CHROMATOGRAMS FOR CHICKEN SKIN PLUS ADHERING FAT



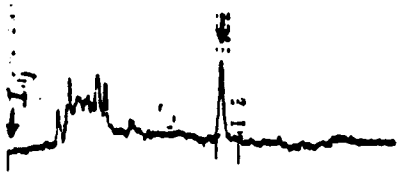
Control skin
100 mg injected
<2 ng CGA-136872
<0.05 ppm



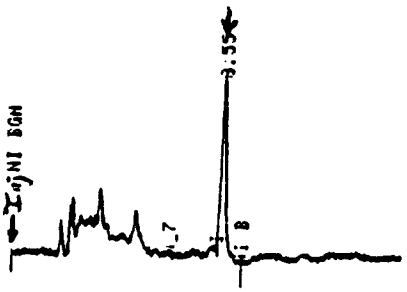
Control + 0.05 ppm
100 mg injected
4.73 ng CGA-136872
95% recovery



Control + 0.05 ppm
100 mg injected
3.90 ng CGA-136872
78% recovery

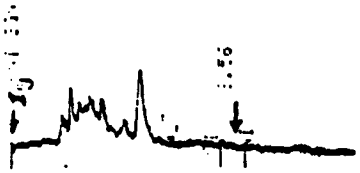


Control + 0.10 ppm
100 mg injected
8.11 ng CGA-136872
81% recovery



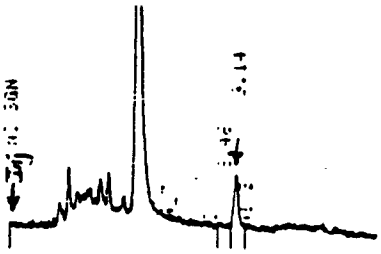
Control + 0.20 ppm
100 mg injected
17.1 ng CGA-136872
86% recovery

FIGURE 12: TYPICAL CHROMATOGRAMS FOR CHICKEN FAT



Control Fat
100 mg injected
<2 ng CGA-136872
<0.05 ppm

Control + 0.05 ppm
100 mg injected
4.21 ng CGA-136872
84% recovery

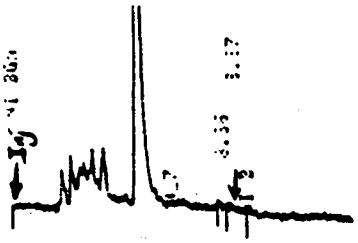


Control + 0.05 ppm
100 mg injected
4.64 ng CGA-136872
93% recovery

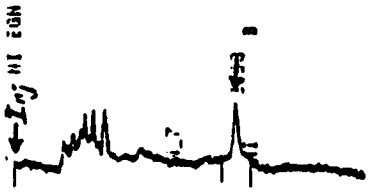
Control + 0.10 ppm
100 mg injected
9.67 ng CGA-136872
97% recovery

Control + 0.20 ppm
100 mg injected
18.6 ng CGA-136872
93% recovery

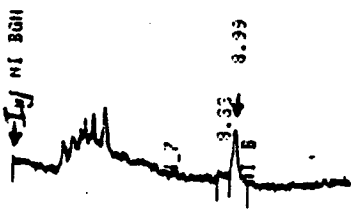
FIGURE 13: TYPICAL CHROMATOGRAMS FOR EGGS



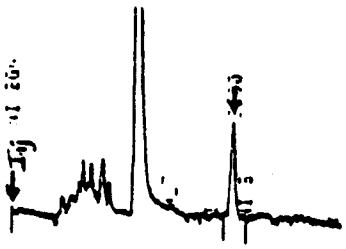
Control Eggs
93.1 mg injected
<2 ng CGA-136872
<0.05 ppm



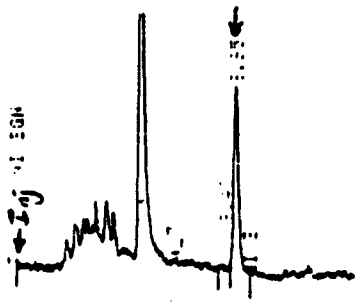
Control + 0.05 ppm
93.1 mg injected
4.20 ng CGA-136872
90% recovery



Control + 0.05 ppm
93.1 mg injected
3.68 ng CGA-136872
79% recovery



Control + 0.10 ppm
93.1 mg injected
8.06 ng CGA-136872
87% recovery



Control + 0.20 ppm
93.1 mg injected
16.5 ng CGA-136872
89% recovery

VII. REFERENCES

1. AG-506, Beidler, W. T. and K. P. Shoffner, "Determination of CGA-136872 in Dairy and Poultry Tissues, Eggs and Milk by High Performance Liquid Chromatography".

2. ABR-85076, Anderson, W. A., S. O. Madrid, and J. E. Cassidy, "Metabolism of Phenyl-^{14}C-CGA-136872 by a Lactating Goat Dosed at 4 ppm for Eleven Consecutive Days".

3. BIOL-85009, Seim, V. W. and J. Burgener, "Metabolism of ϕ-^{14}C-CGA-136872 in a Goat at 4 ppm."

4. SOP No. 4.67, Torbett, M., "Operation, Maintenance and Calibration of Manual Harvey OX-400 Oxidizers".

5. SOP No. 4.60 (Rev. 1), Beidler, W. T., "Procedures for Operation, Calibration, Maintenance and Documentation of the Beckman Model 7800 Liquid Scintillation Counter."

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PAGE 1 of 5	METHOD NO. AG-506B	SUBJECT
EDITION 10/4/88	AG-506B (supersedes AG-506A)	ADDENDUM TO AG-506: SUBSTITUTION OF ACETONITRILE FOR METHANOL FOR EXTRACTION OF CGA-136872 RESIDUES FROM MILK
SUBMITTED BY: K. Van Geluwe-Barvir, T. Beidler		
		APPROVED BY:

1.0 SCOPE

This addendum describes the substitution of acetonitrile for methanol used in AG-506, "Determination of CGA-136872 in Dairy and Poultry Tissues, Eggs and Milk by High Performance Liquid Chromatography," for extraction of CGA-136872 residues from milk. This addendum supersedes and replaces AG-506A. Both solvents are equally effective in extracting CGA-136872 but acetonitrile eliminates coextractives encountered with milk that cause emulsions when methanol is used. The use of diatomaceous earth is not necessary when extracting with acetonitrile. The extraction is performed by shaking for 20 minutes on a mechanical shaker.

This alternative solvent for extraction is a direct substitution. The ratio of acetonitrile:water remains the same as methanol:water at 9:1.

2.0 PRINCIPLE

The entire method showing the substitution of 90% acetonitrile/water for 90% methanol/water as the extracting solvent is outlined in Figure 1. Additional procedural details have been added to the pertinent Sections of AG-506 to provide better descriptions of the results using acetonitrile.

Only those sections in AG-506 dealing specifically with the extraction and the discussion of results are listed in this addendum. The corresponding numbering system of AG-506 is used.

3.0 APPARATUS

3.18 Mechanical Shaker

4.0 REAGENTS

4.20 Acetonitrile:distilled water, 9:1.

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SUBMITTED BY: K. Van Geluwe-Barvir, T. Beidler		
		APPROVED BY:

5.0 PROCEDURE

5.2 Extraction

5.2.1 Milk

5.2.1.1 Weigh a 20-gram subsample of milk into a 16-oz. square amber glass bottle.

5.2.1.2 Add 200 ml of acetonitrile:water (9:1) extraction solvent. Cap bottle and shake for 20 minutes on a mechanical shaker.

5.2.1.3 Filter the extract through Whatman 2V filter paper and collect the filtrate in an 8 oz. Boston round bottle.

5.2.1.4 Proceed directly to Step 5.3.1 of AG-506 and continue as described therein.

9.0 DISCUSSION (of AG-506B)

9.1 Acetonitrile and H_2O (9:1) has been used as an alternative solvent for extraction of milk samples analyzed by AG-506. Representative HPLC chromatograms are illustrated in Figure 2.

Recovery data for CGA-136872 using this extraction technique are given below:

% Recovery at Various Fortifications of CGA-136872

Substrate	0.01 ppm	0.05 ppm	0.10 ppm	0.50 ppm
Dairy Milk	84,84,89,97,98	95	90,97,103,103	95,96,99

No apparent residues (<0.01 ppm) were observed in the unfortified samples.

9.2 No difference in appearance of HPLC chromatograms was observed using acetonitrile:water (9:1) instead of methanol:water (9:1) for extraction of milk.

PAGE 3 of 5	METHOD NO. AG-506B	SUBJECT
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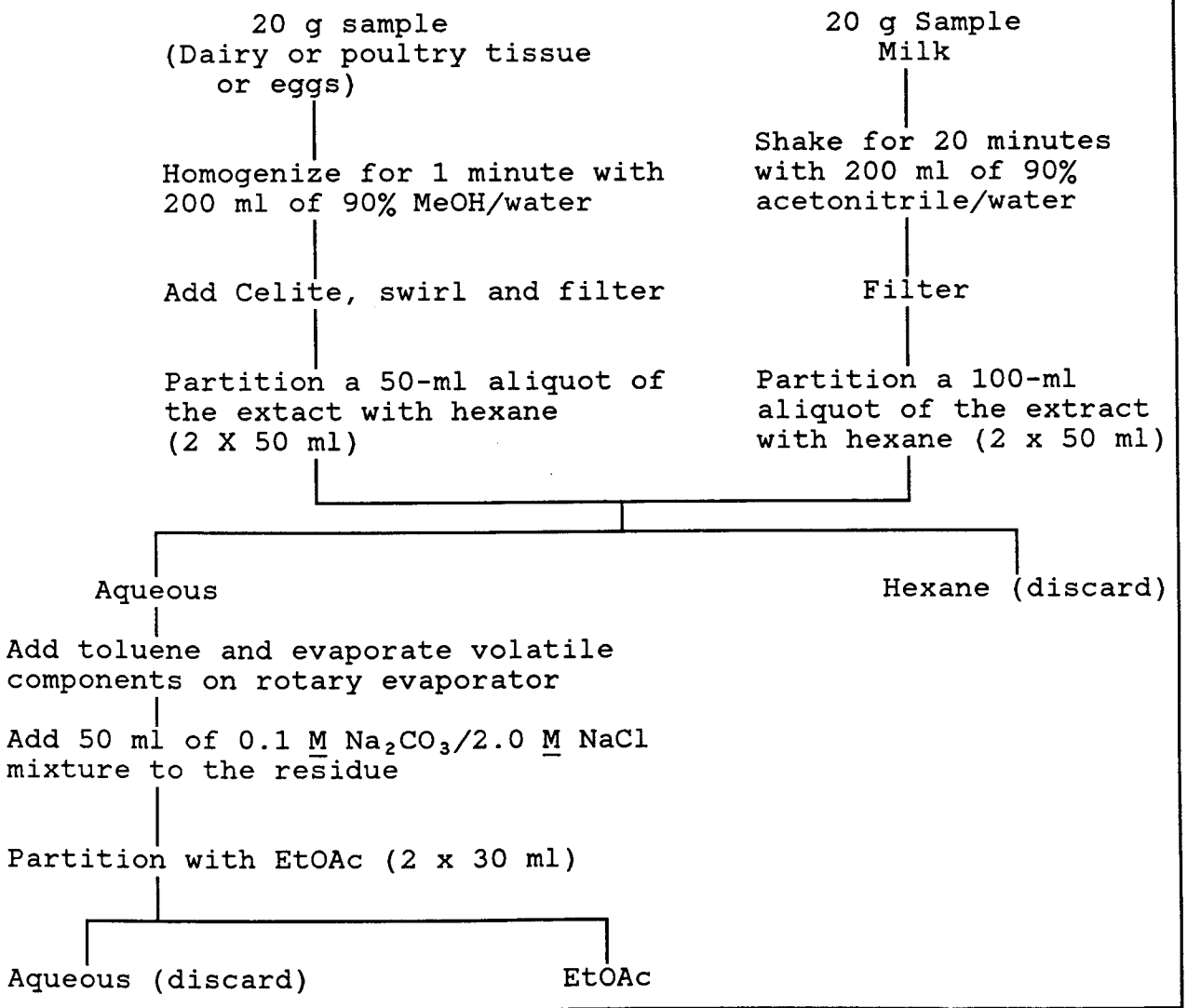
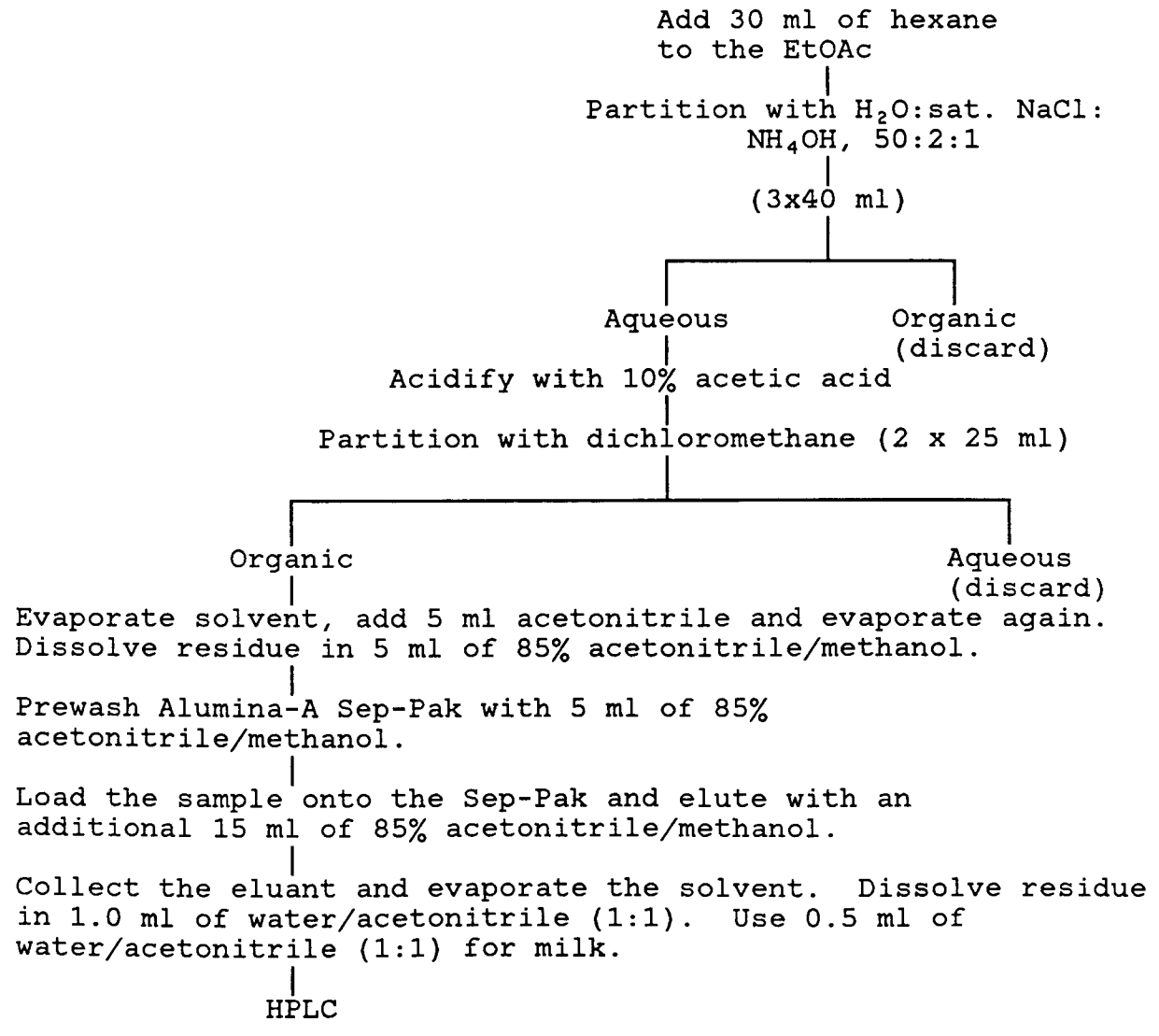
SUBMITTED BY: K. Van Geluwe-Barvir, T. Beidler		
		APPROVED BY:

FIGURE 1: FLOW DIAGRAM FOR ANALYTICAL METHOD FOR DETERMINATION OF CGA-136872 IN DAIRY AND POULTRY TISSUES, EGGS, AND MILK BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY



PAGE 4 of 5	METHOD NO. AG-506B	SUBJECT
EDITION	AG-506B (supersedes AG-506A)	ADDENDUM TO AG-506: SUBSTITUTION OF ACETONITRILE FOR METHANOL FOR EXTRACTION OF CGA-136872 RESIDUES FROM MILK
SUBMITTED BY: K. Van Geluwe-Barvir, T. Beidler		
		APPROVED BY:

FIGURE 1: FLOW DIAGRAM FOR ANALYTICAL METHOD FOR DETERMINATION OF CGA-136872 IN DAIRY AND POULTRY TISSUES, EGGS, AND MILK BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (Continued)



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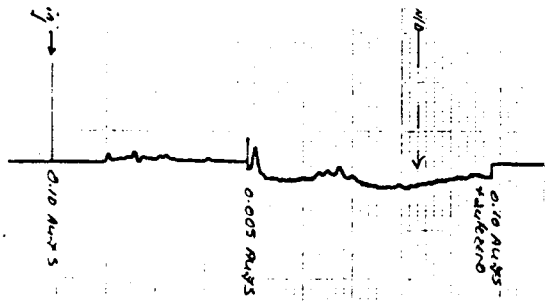
PAGE 5 of 5	METHOD NO. AG-506B	SUBJECT
EDITION	AG-506B (supersedes AG-506A)	ADDENDUM TO AG-506: SUBSTITUTION OF ACETONITRILE FOR METHANOL FOR EXTRACTION OF CGA-136872 RESIDUES FROM MILK
SUBMITTED BY: K. Van Geluwe-Barvir, T. Beidler		
		APPROVED BY:

FIGURE 2: TYPICAL CHROMATOGRAMS FOR MILK SAMPLES (AG-A 10242)



Control Milk
368 mg injected
<2 ng CGA-136872 found
<0.01 ppm

Control + 0.10 ppm
92 mg injected
8.9 ng CGA-136872 found
97% recovery

Treated sample
4-7-A
368 mg injected
8.5 ng found
0.02 ppm